

N70 31845

NASA TECHNICAL  
MEMORANDUM

NASA TM X- 58045  
June 1970



THE DEVELOPMENT AND APPLICATION OF PLETHYSMOGRAPHIC  
AND ISOTOPIC METHODS FOR STUDYING THE RELATIONSHIP  
BETWEEN INTRINSIC AUTOREGULATION OF BLOOD FLOW AND  
THE PARTITION OF INTERCOMPARTMENTAL FLUID  
VOLUMES IN SKELETAL MUSCLE

A Thesis Submitted to  
the Graduate Faculty of  
Wake Forest University  
In Partial Fulfillment of  
the Requirements for the Degree  
Doctor of Philosophy  
in the Department of Physiology of  
The Bowman Gray School of Medicine

CASE FILE  
COPY

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION  
MANNED SPACECRAFT CENTER  
HOUSTON, TEXAS

NASA TM X-58045

THE DEVELOPMENT AND APPLICATION OF PLETHYSMOGRAPHIC  
AND ISOTOPIC METHODS FOR STUDYING THE RELATIONSHIP  
BETWEEN INTRINSIC AUTOREGULATION OF BLOOD FLOW AND  
THE PARTITION OF INTERCOMPARTMENTAL FLUID  
VOLUMES IN SKELETAL MUSCLE

William Carter Alexander  
Manned Spacecraft Center  
Houston, Texas

## ACKNOWLEDGMENTS

I would like to extend my sincere gratitude to Professor Harold D. Green, a true scholar, teacher, and friend, for the opportunity to study and work under his capable direction.

I am grateful to Dr. Robert F. Bond, who, in addition to giving so unselfishly of his time to guide my dissertation research, always demonstrated a keen appreciation of the philosophy of a graduate education.

To Drs. E. M. Renkin, F. L. Thurstone and Phillip C. Johnson whose encouragement and constructive criticism has given my program real meaning, I am most appreciative.

I express my sincere thanks to Miss Rosalind Frank for her able technical assistance in conducting the experiments and for the preparation of the illustrations in the text. Additionally, I thank Mr. Keith Fischer, Mrs. Susan Goodridge, and Miss Theda Driscoll for their individual contributions to the project, and Mrs. Anne Mathews and Mrs. Gay Sultan for the preparation of the final manuscript.

Without the generous support of the North Carolina Heart Association, the National Heart Institute, USPHS (HE-00487), the Cardiovascular Graduate Training Program (HE-5392), and

the National Aeronautics and Space Administration (NAS 9-8949),  
this program would have not been possible.

## TABLE OF CONTENTS

	Page
List of Illustrations	vii
Abstract	ix
INTRODUCTION	1
A. General	1
B. Historical Review	4
1. Animal studies	4
2. Methodology	7
a. Plethysmograph	7
b. Measurement of intravascular volume	10
C. The Present Study	13
MATERIALS AND METHODS	14
A. Procedural Aspects	15
1. General	15
2. Surgical isolation of gracilis muscle	16
3. Catheter design	18
4. Plethysmograph design	22
B. Technical Measurements (Data Acquisition)	26
1. Pressure-flow	28
2. Compartmental Fluid volumes	30
C. Protocol and Data Analysis	33

	Page
RESULTS	35
DISCUSSION	45
A. Introduction	45
B. Critique of Biological Preparation	46
C. Experimental Evidence	48
D. Conclusions	54
APPENDIX A	55
Design Considerations and Specification of the Plethysmographic Chamber	
APPENDIX B	64
Physical Characteristics of the System Employed for the Measurement of Intravascular Volume by $^{51}\text{Cr}$ -RBC Dilution	
APPENDIX C	73
Glossary and Calculation of Intravascular Volume from Saline Washout	
1. Glossary	73
2. Calculations	74
BIBLIOGRAPHY	76
SCHOLASTIC VITA	82
REPRINT	84

## LIST OF ILLUSTRATIONS

	Page
Figure 1. Arterial and venous extracorporeal catheter circuits for vascular perfusion.	20
Figure 2. Photograph of complete catheter circuit in place with isolated gracilis muscle <u>in situ</u> .	21
Figure 3. Photograph of gracilis muscle in the plethysmographic chamber.	24
Figure 4. Schematic diagram of instrumentation employed for measurement of vascular parameters.	27
Figure 5. Total muscle volume, gracilis vein blood flow, and vascular conductance during a stepwise reduction of perfusion pressure.	36
Figure 6. Comparison of pressure/flow plots in dilated and constricted beds.	39
Figure 7. Total muscle volume responses recorded during stepwise reduction of perfusion pressure.	40
Figure 8. Total muscle volume, intravascular volume, and vascular dynamics recorded during stepwise reduction in perfusion pressure.	42
Figure 9. Intravascular volume and extravascular volume during stepwise reduction in perfusion pressure.	44
Figure 10. Photograph of the plethysmograph chamber.	56
Figure 11. Dimensions of the plethysmograph chamber.	57
Figure 12. Dynamic response of the plethysmograph recorded by rapid injection of 1.0 cm <sup>3</sup> of mineral oil.	60

	Page
Figure 13. Dynamic response of the plethysmograph recorded by rapid withdrawal of 1.0 cm <sup>3</sup> of mineral oil.	60
Figure 14. Photograph of the scintillation detector above plethysmograph.	65
Figure 15. Schematic diagram of instrumentation employed for detection and registration of the activity of <sup>51</sup> Cr.	66
Figure 16. Lateral view of scintillation detector over plethysmograph describing the areas subtended by the penumbra, the total solid angle, and the crystal circumference in relation to the mass of the gracilis muscle.	67
Figure 17. Top view of surface areas subtended by the penumbra, the total solid angle, and the crystal circumference in relation to the mass of the gracilis muscle.	68
Figure 18. The physical relationship between the object and scintillator assembly considered in the derivation of the loss of counting efficiency due to geometric considerations.	70
Figure 19. Catheter circuit and detector efficiency as a function of distance from the center of the muscle.	71



THE DEVELOPMENT AND APPLICATION OF  
PLETHYSMOGRAPHIC AND ISOTOPIC METHODS  
FOR STUDYING THE RELATIONSHIP BETWEEN  
INTRINSIC AUTOREGULATION OF BLOOD FLOW AND  
THE PARTITION OF INTERCOMPARTMENTAL  
FLUID VOLUMES IN SKELETAL MUSCLE

William Carter Alexander

Research Advisers: Professor Harold D. Green

Professor Robert F. Bond

Cardiovascular volume homeostasis is the result of the integration of both systemic (extrinsic) and local (intrinsic) reflex mechanisms. Extrinsic and intrinsic modulation of the peripheral vasculature, and, in particular, the vascular beds of skeletal muscle, in part governs the partition of fluid between the intravascular and extravascular compartments. The relationship between purely intrinsic vascular responses and the transfer of fluids in the capillary circulation suggests that autoregulation of blood flow in skeletal muscle may also result in a similar regulation of capillary hydraulic pressure. If this regulation does exist in the vasculature of skeletal muscle, such a mechanism would serve to decrease the effective systemic vascular volume by the retention of significant volumes

of intravascular and extravascular fluids in the peripheral vascular beds of skeletal muscle.

An oil-filled, volume plethysmograph was designed to study the magnitude, direction, and temporal sequence of intercompartmental fluid exchange in relation to intrinsic, autoregulatory responses of the peripheral vasculature. The denervated canine gracilis muscle was dissected from the hindlimb and placed in the plethysmographic chamber. The gracilis artery and vein were reconnected to the femoral circulation of the animal through a low resistance extracorporeal catheter circuit. Blood flow was measured by a cannulating-type, electromagnetic blood flow probe (Carolina Medical Electronics, Inc.) and perfusion pressure, by appropriate transducers. The proximal connection to the animal and the vascular "seal" of the plethysmograph were established through the non-collapsible lateral ports of the blood flow probes. The pressure analog of muscle volume change was registered by a transducer placed in series with the chamber and a constant pressure reservoir. With radio-labeled RBC's ( $^{51}\text{Cr}$ ) used as indicator, the segmental volume of the vascular space was obtained from the ratio of the activity of labeled blood in the saline washout to the activity of the perfusing blood. Sequential changes in the size of the intravascular space were recorded by

monitoring continuously the activity of the muscle with a 1.5 inch (3.8 cm) open-bore, collimated scintillation detector. This activity was then scaled to reflect the size of the intravascular volume at the time of saline washout. The relative size of the extravascular compartment was computed as the numerical difference between the total muscle and intravascular volumes.

The changes in the magnitudes of these fluid compartments were compared simultaneously with the pressure-flow relationships existing across the vascular bed in twenty experiments.

The results of this investigation demonstrate that the changes in total muscle volume paralleled changes in vascular conductance through the range of perfusion pressure from 140 - 70 mm Hg; whereas, below this range, muscle volume fell markedly without a corresponding decrease in conductance. Changes in the intravascular volume followed a similar pattern. An apparent stabilization of intravascular volume in skeletal muscle was evident through the range of perfusion pressure between 90 - 60 mm Hg; this finding suggested a reabsorption of fluid from the extravascular compartment and a reduced rate of decrease of the intravascular volume as the perfusion pressure was decreased from 90 to 60 mm Hg. Below 60 mm Hg perfusion pressure, intravascular, extravascular, and total

muscle volumes decreased more rapidly relative to this response recorded at perfusion pressures between 90 - 60 mm Hg.

These changes in the magnitude and direction of intercompartmental fluid shifts recorded in this study suggest that through the autoregulatory range of blood flow in skeletal muscle, a mechanism does exist whereby intravascular volume is stabilized by a regulatory modulation of capillary hydraulic pressure. The potential contribution of this local mechanism to the maintenance of the systemic blood volume is yet to be described.

## INTRODUCTION

### A. General

The ability of the systemic circulation to regulate its volume has intrigued the student of circulatory physiology for many years. It is generally appreciated that the systemic capacitance reservoir (the vena cavae and small veins) preserves an optimal balance between the volume of blood contained within and the total capacity of the system. It is this balance which ultimately defines cardiac filling pressure, and, thereby, the output of the heart (18, 24, 25, 29). Both systemic and local mechanisms function to regulate the capacity of the circulatory reservoir within relatively narrow limits. Numerous original articles (30, 35, 40, 51) and reviews (15, 23, 42), which describe the systemic and peripheral components of the reflex control of vascular capacity and volume, have appeared in the scientific literature. The fact that the integrated response of these two principal mechanisms is essential for the preservation of circulatory homeostasis has long been established.

The relationship between the vascular dynamics, which exists in the various parallel and series-coupled segments of the

local tissue circulation, and the partition of fluids between the vascular and extravascular compartments, is of particular importance in the peripheral aspects of this regulation. The local responses while not the sole aspect of peripheral control, nevertheless provide a modulator influence on the integrated performance of the system. A thorough appreciation of this intrinsic behavior is essential to the understanding of the overall contribution of peripheral control to cardiovascular homeostasis. In conjunction with these principles, this dissertation concerns: a) the development of a plethysmographic technique to describe the magnitude, direction, and temporal sequence of intercompartmental fluid exchange, and b) the application of this method to study the relationship between intrinsic hemodynamics and solvent exchange in mammalian skeletal muscle.

The vascular bed of skeletal muscle serves as an "ideal" biological model to study the relationship between local circulatory dynamics and compartmental fluid exchange. This premise is based on the observation that the dynamic responses of the innervated vascular bed of skeletal muscle are modulated by both extrinsic (neurogenic and neurohumoral) (12, 20, 21, 39) and intrinsic (autoregulatory) (14, 28, 44, 45) factors. This response in denervated preparations is, however, governed by local chemical

and physical factors which influence exchange and support functions (7). Since skeletal muscle comprises approximately 40% of the mammalian body weight and contains nearly 50% of the total body water within its structure (43), it seems reasonable to assume that the skeletal muscle mass and its vasculature represent a significant potential source and/or sink for the redistribution of body fluids. The mobilization of this fluid volume is governed, at least in part, by intrinsic vascular responses at the tissue level. Autoregulation of blood flow in the intact animal serves both the support and exchange functions of the peripheral circulation. In a denervated vascular bed, however, the exchange function of autoregulation may be predominant. Autoregulation of flow functions primarily to preserve the nutrient supply to meet the metabolic demands of the tissues, and may also offer at the same time a means of stabilizing capillary hydraulic pressure. Consequently, if this mechanism does exist in skeletal muscle, compartmental fluid exchange may also be "autoregulated." Numerous investigators (13, 16, 35) have alluded to this possibility; however, few, if any specific tests of this hypothesis have appeared in the literature. It also remains to be shown whether such a regulatory response is beneficial or detrimental to the organism.

## B. Historical Review

### 1. Animal studies.

Öberg (35), in a rigorous and comprehensive study of the effect of cardiovascular reflex mechanisms on the net capillary fluid exchange, demonstrated the existence of a wide range of control exercised over the pre- and post-capillary resistive segments of the vasculature in muscle, skin, and intestine. This investigator maintained arterial pressure essentially constant, but varied the efferent nervous input to the vascular beds by stimulating the carotid sinus baroreceptor or the aortic and carotid chemoreceptor areas. Öberg's work clearly demonstrated the extent to which fluid mobilization is governed by extrinsic modulation of the vasculature. A critical analysis of his reported data suggests that in the presence of autoregulation of blood flow, the tissue preparations either gained or lost volume as a function of the resultant "set" of the pre-postcapillary resistance ratio. This finding may be interpreted to mean that in the intact animal, autoregulation of blood flow does not necessarily imply stabilization of capillary hydraulic pressure, a situation which would prevent a net exchange of fluid; but instead strongly suggests that both extrinsic and intrinsic factors act synergistically to compliment the overall requirements for



solvent and solute exchange.

Further supportive evidence that a balance of extrinsic and intrinsic modulation exists between capillary exchange and autoregulation of blood flow is apparent in a report by Gregersen (22) who observed that following prolonged bilateral carotid occlusion which results in a substantial increase in systemic pressure, no decrease in circulating blood volume occurred. It could be predicted that if the pre-post capillary resistance ratio were constant, a large increase in arterial pressure would produce a net outward filtration of fluid from the vascular space and, consequently, a lowering of the blood volume. Gregersen's data do not support this prediction, but rather suggest that capillary pressure did not rise in proportion to the increase in arterial pressure. This finding can be explained by a substantial increase in the pre-post capillary resistance ratio. Data reported by other investigators (34, 35, 37) justify this interpretation by demonstrating that vasoconstrictor fiber stimulation causes an increased precapillary resistance in the intact animal.

It appears that in the intact animal, the nutrient flow to the tissues and the magnitude of capillary pressure are regulated separately, even though their performances are complementary. A question now arises concerning the relationship between the

two factors when extrinsic modulator influences are abated or absent.

In 1961, Folkow and Öberg (13) reported the results of a study designed to verify the hypothesis that autoregulation and basal vascular tone are inherent to the smooth muscle cell, the function of which controls the precapillary resistance vessels. These workers also presented data which suggest that during autoregulation in a "denervated" vascular bed, precapillary resistance changed in such a way as to "protect" the capillary bed from large increases or decreases in arterial pressure. Consequently, capillary hydraulic pressure was essentially stabilized by the upstream resistance, implying no net change in the extravascular volume of the tissue. Their data, recorded from reserpinized cats which lacked extrinsic control over selective resistance sites in the microcirculation, drew attention to the fact that the intrinsic or autoregulatory response of the vasculature may well control capillary pressure. Renkin (38) has presented data supporting this possibility in studies which show that during autoregulation the capillary transport coefficient,  $PS$  (product of capillary permeability and surface area available for exchange of solute or solvent), is stabilized over the same range of pressures as is the blood flow.

Accordingly, it appears that autoregulation maintains intravascular pressure constant at the level of the arterial capillary. In the absence of extrinsic modulator influence, the capillary hydraulic pressure becomes a function of the degree of precapillary vasomotor tone (arteriolar resistance) and of the flow through the capillary bed (3). The precapillary sphincters which respond to local chemical stimuli govern the partition of the available flow between the capillary bed and non-nutritive vascular channels (37, 38, 47). In this manner, autoregulation controls the capillary pressure, and, thereby, solvent exchange. Therefore, autoregulation of fluid exchange at the capillary level is proposed as a hypothesis for the maintenance of intravascular volume and the preservation of circulatory homeostasis.

## 2. Methodology.

a. Plethysmography. Since Brodie and Russell (8) first described a means of measuring blood flow from an organ placed in an oncometer, the fluid displacement plethysmograph has been used widely in peripheral circulatory studies. The majority of these applications has been employed to study segments of a total extremity (e. g., a foot, arm, or calf, rather than the extremity as a unit) to obtain specific information regarding vascular responses of an isolated segment of the

body (49). Studies on the human forearm (5) and the "isolated" hindquarters of laboratory animals (34, 35) have provided data concerning regional vascular and fluid transport mechanisms.

Mellander (34) devised a plethysmographic method whereby the reactions of the pre- and post-capillary resistance vessels, and the capacitance vessels could be recorded separately in the "isolated" hindlimb of a cat. He evaluated his results on the basis of their different response times following experimental manipulation of the preparation. Mellander's plethysmograph was a fluid-filled chamber into which the animal's hindquarters were secured. The nervous and vascular connections to the animal were passed through a skin-flap seal near the umbilicus. Volume changes in the various fluid compartments were registered on a piston-recorder. The basic principles of Mellander's technique have been employed widely by his colleagues (1, 9, 35) in a large number of studies concerning the relationship between cardiovascular dynamics and intercompartmental fluid transfer. Collectively, these workers have described, in considerable detail, the various interactions between extrinsic and intrinsic modulation of the fluid transport mechanisms in muscle, skin, and intestinal preparations.

Although a large number of investigators have applied the

technique of sectional fluid displacement plethysmography for the study of fluid transport mechanisms, there have been no significant changes in the basic design of the plethysmographic chamber. These chambers reflect design considerations which are essential to maintaining a non-restrictive, physiological environment for the organ under study, while providing the stability and sensitivity to register accurately the physiological variations. When differences between methodologies occur, they are related generally to the means by which the vascular supply channels are protected from hydrostatic pressure effects arising from pressure-volume changes within the chamber. In the majority of cases, the effective "seal" of the supplying vasculature has been a limiting factor in the use of the method. Secondly, the means by which volume changes are recorded differ. Since a pressure recording does not allow for expansion and contraction of the fluid phase of the chamber, hydrostatic loading of the vasculature or of the total organ has made this method unacceptable. Consequently, most approaches have employed an air phase in which the pressure changes are reflected as an analog of organ volume change. This air phase either is continuous with a piston assembly which displaces an appropriate volume of a second fluid, which is not a part of the plethysmograph, or with an air-filled dome of a

pressure transducer. No significant hydrostatic pressure would be reflected on the contents of the chamber if the piston or the diaphragm of the pressure transducer were highly compliant.

In the plethysmograph designed for the present study, the problems of an adequate, yet non-restrictive, vascular "seal" have been eliminated by employing the non-collapsible flow ports of an electromagnetic blood flow probe as the proximal connection between the animal and the vascular supply to the muscle within the chamber. Hydrostatic back-pressure was minimized by placing a constant pressure reservoir (oil/air phases) between and in series with the chamber and the diaphragm of an electrical pressure transducer.

b. Measurement of intravascular volume. The measurement of regional intravascular volume and changes in this volume concomitant with skeletal muscle hemodynamics has been attempted largely by two methods. First, by accurately determining the blood flow and the mean transit time of an indicator through a vascular bed, the segmental volume of the traced circuit may be calculated from the product of the two parameters (50). This procedure has received much attention in the past few years (4, 19); however, the validity of the method for measurement of segmental volume in peripheral vascular beds

is currently unresolved. A second approach to the measurement of segmental vascular volume is based on the dilution principle (46), in which an unknown volume can be described as the ratio of the amount of injected indicator remaining within the unknown space or volume to its mean concentration at equilibrium. Both methods yield information concerning the size of the intravascular space only at the time of the determination. Continuous sampling of the indicator concentration for serial determinations of vascular volume is limited by recirculation of the indicator substance, and by residual indicator in the vascular space from a previous determination. However, by using an isotopic indicator substance and continuously detecting the activity of the isotope in the vascular space, relative changes in the segmental volume can be recorded as a function of time. By washing out the vascular space with saline or dextran, and accurately determining the activity of the effluent volume (36), the temporal registration of this activity can be related to an actual measurement of vascular volume. The ratio of the total activity of the washout to the activity per unit volume of perfusing blood is recorded as the segmental volume of the vascular space. Temporally recorded values of the isotopic activity, which represent sequential changes in the vascular volume, are scaled

to reflect the size of the intravascular space at washout.

Baker and O'Brien (4) reported the results of a study which compared the recorded values of intravascular volume determined by  $^{51}\text{Cr}$ -labeled red blood cells,  $^{131}\text{I}$ -labeled albumin, Indocyanine, and changes in limb weight. These investigators concluded that all indicators employed gave reliable results for large changes in vascular volume. The  $^{51}\text{Cr}$  red blood cell derived volumes, however, were consistently lower than those recorded by the other indicator substances or by changes in limb weight. The basis for this finding is questionable and is still being debated.

In a series of experiments using the cat hindlimb, Mellander (34) suggested that regional vascular and tissue volume changes could be measured accurately using the plethysmographic recording method, provided the changes in vascular responses developed rapidly. In a later study, comparing the effects of various dilator substances on a similar preparation, Åblad and Mellander (1) employed for the measurement of regional volume a  $^{51}\text{Cr}$ -red blood cell ( $^{51}\text{Cr}$ -RBC) dilution method in conjunction with a plethysmograph. In this investigation, the changes in segmental volume recorded by the isotope monitoring method correlated well with those registered by the plethysmograph.



In the present research, no correlation can be made between the intravascular volume derived isotopically and the total muscle volume registered by the plethysmograph because the time course of the induced vascular responses is too great for separation of the components of the plethysmographic record. In view of this fact and the continuing debate over the accuracy of the  $^{51}\text{Cr}$  red blood cell dilution method as a means of measuring the regional intravascular volume, all references to "intravascular volume" in this dissertation imply only that volume derived from the  $^{51}\text{Cr}$  red blood cell dilution space.

### C. The Present Study

This dissertation is founded on the hypothesis that the intrinsic response of the peripheral tissue vasculature operates in opposition to the systemic circulation, and is a principal contributor to the deterioration of cardiovascular integration and circulatory homeostasis at low perfusion pressures. To investigate this hypothesis, it was necessary to establish a method of approach; develop and perfect a technique for the registration of cardiovascular parameters; and, through appropriate manipulation of the peripheral circulation, describe the partition of intravascular and tissue compartmental fluid volumes in skeletal muscle.

## MATERIALS AND METHODS

In studies concerned with the dynamic behavior of vascular beds and, in particular, the complex relationship between circulatory function and compartmental fluid transfer, one must use extreme care in the surgical isolation of the organ for study, and must consider the numerous factors which ultimately influence the physiological reactivity of the preparation. Attention must be paid, also, to the mechanics of recording the analog parameters which reflect the dynamic changes in the preparation.

In the present investigation, attempts were made to control the environmental factors (i. e., temperature and hydration) which may affect the reactivity of the tissue being studied. Specific mention of these controls is found under appropriate headings in the sections that follow.

The investigation was functionally divided into two phases: first, a phase devoted to the development and perfection of a technique for the measurement of compartmental fluid shifts in skeletal muscle utilizing an oil-filled plethysmograph, and a second phase involved the measurement of fluid exchange dynamics between these compartments simultaneously with other

circulatory parameters. The latter aspect applied the derived technique to the study of compartmental fluid transfer in the canine gracilis muscle and also served as an evaluation of the design philosophy of the method.

#### A. Procedural Aspects of the Method

1. General. Forty-seven healthy canine mongrels, chosen without regard to sex, with body weights varying between 9.0 - 12.0 kg were employed as experimental animals. Surgical anesthesia was induced with sodium pentobarbital. An initial dose of 30 mg/kg of body weight was administered intravenously, and supplemental doses of 0.5 - 1.0 ml were given throughout the experiment as needed to maintain surgical anesthesia. The depth of anesthesia was judged on the presence or absence of a corneal blink reflex. Lower level plane II of stage III anesthesia was considered optimum from the standpoint of muscle relaxation and reflex tone (11). An endotracheal tube was inserted, and the anterior surface of both hindlimbs shaved from the umbilicus to the paw. Body temperature was monitored using a rectal probe which, when connected through a telethermometer, thermostatically regulated a heating pad and maintained the animals' core temperature at  $37.5^{\circ}\text{C} \pm 0.1$ . Large bore polyethylene tubing was introduced into the left

femoral vein for supplemental infusion of anesthesia and administration of drugs. Throughout the operative phase of the experiment, physiological saline was infused at a very slow rate to compensate for bleeding and evaporative  $H_2O$  loss from the respiratory tract and exposed tissues.

2. Surgical isolation of gracilis muscle (26, 33). An incision was made extending distally from the inguinal ligament to the knee joint and medially across the anterior surface of the adductor muscles of the right hindlimb with the resulting skin flap retracted medially to expose the anterior muscles of the thigh. Using an electrocautery (National, Model 37), and blunt dissection, the gracilis muscle was then carefully dissected from its superficial and deep fascial components. All observable vessels in the muscle sheath and skin flap were doubly ligated and divided. The fascial components of the tendons were stripped from the muscle belly to the bony attachments. Extreme care was taken to protect the muscle from undue trauma during the entire dissection. To minimize evaporative drying, the muscle was covered at all times with a thin sheet of clear plastic and bathed in warm saline. Temperature of the muscle was maintained at  $37.5^{\circ}C \pm 0.1$  by means of a servo-thermometer which operated a 150 watt lamp placed over the

preparation.

The gracilis artery and vein were carefully dissected from the femoral vessels to the gracilis muscle. All vessels arising from the gracilis vessels were doubly ligated and divided. The gracilis branch of the obturator nerve was dissected for a distance of 3 - 4 cm from the muscle at which point it was ligated and divided. In animals of the weight range chosen for this study, small vascular twigs originating from the medial saphenous vessels and supplying the distal aspect of the gracilis muscle were either nonexistent or of such small size that they were considered an insignificant portion of the vascular supply to the tissue under study. These twigs were also ligated and divided routinely. The surgical procedure resulted in a total isolated, denervated, skeletal muscle preparation, with a single supplying artery and draining vein.

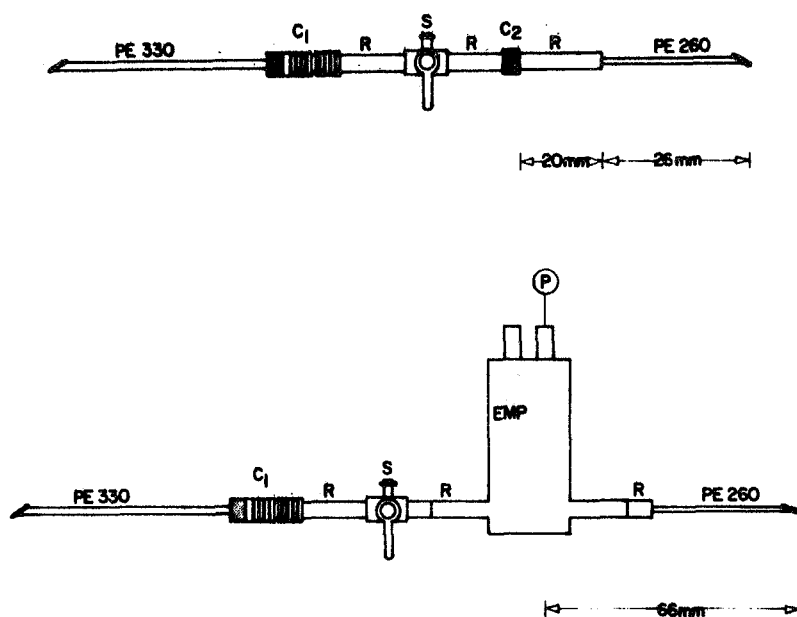
The dissection was completed by freeing the femoral artery and vein from their fascial attachments over their entire length from the inguinal ligament to the popliteal bifurcation. With the exception of the gracilis artery and vein which were left patent for perfusion of the isolated muscle, all vessels arising from the femoral vessels between the inguinal ligament and medial saphenous branches were ligated and divided. The two tendons

were separated into a series of small sections and bound with double ligatures.

At the conclusion of the dissection, the animal was heparinized (5 mg/kg of body weight) and left undisturbed for a period of one hour to recover from the operative trauma. Supplemental heparin (one-half of initial dose) was given every 45 minutes. At all times, the temperature of the gracilis muscle was maintained constant, by wrapping the muscle in saline-soaked gauze and covering it with a plastic drape.

3. Catheter design (Figs. 1 and 2). A series of experiments was conducted to select a catheter circuit which offered the least trauma to the excised vessels, minimal impetus to clot initiation, and the least resistance to blood flow. In addition, the catheter system required a means of rapid disconnection and reconnection to facilitate the transfer of the gracilis muscle from the hindlimb to the plethysmograph. One of the circuit designs discarded was a small bore polyethylene segment which was inserted into the gracilis artery and vein from openings in the femoral vessels. This approach was abandoned because these small catheters were extremely difficult to advance through the vasculature without trauma and invariably resulted in vascular spasm and a reduced blood flow. Secondly, these catheters added a significant

mechanical resistance to flow and frequently became occluded with fibrin strands or intimal epithelial cells. A more successful method of catheterization was the securing of larger bore canulae into the femoral vessels at the level of the femoral-gracilis bifurcation with the femoral vessels being ligated distal to their gracilis branches. The catheter circuit (Figs. 1 and 2) included large bore sections constructed from lengths of polyethylene and gum rubber tubing previously treated with silicone (Siliclad, Clay-Adams). The complete catheter circuit, both for artery and vein, consisted of a segment of PE 330 polyethylene tubing joined through a stainless steel adapter to a length of gum rubber tubing fitted with a male/male tubing connector. A three-way plastic stopcock was inserted in the middle of the latter segment. The open end of the male tube connector was mated to a second piece of gum rubber tubing which terminated in a measured length of PE 260 polyethylene. The tip was beveled to facilitate insertion in the femoral vessel and to aid in securing it against the orifice of the femoral-gracilis bifurcation, thus providing minimal resistance to blood flow. The arterial catheter routinely was inserted first in order to re-establish flow to the gracilis muscle in the shortest possible time (usually less than 20 sec). The venous catheters were similarly



## LEGEND

PE 330 - Polyethylene I.D. .115 inch (.292 cm.) CLAY-ADAMS INTRAMEDIC<sup>®</sup>  
 PE 260 - Polyethylene I.D. .070 inch (.178 cm.) CLAY-ADAMS INTRAMEDIC<sup>®</sup>  
 C<sub>1</sub> - Plastic Tubing Adapter  
 C<sub>2</sub> - Male-Male Adapter  
 R - Gum Rubber tubing I.D. .125 inch (.318 cm.)  
 S - Stopcock, 3 way, Plastic  
 EMP - Electromagnetic Blood Flow Probe Model EMP 300P2 Carolina Medical Electronics, Inc.  
 P - Pressure Transducer Statham Instruments, Inc. P 23

Fig. 1. Arterial and venous extracorporeal catheter circuits for vascular perfusion. Upper diagram describes the complete circuit; lower diagram depicts the use of lateral ports of blood flow probes for vascular "seal".



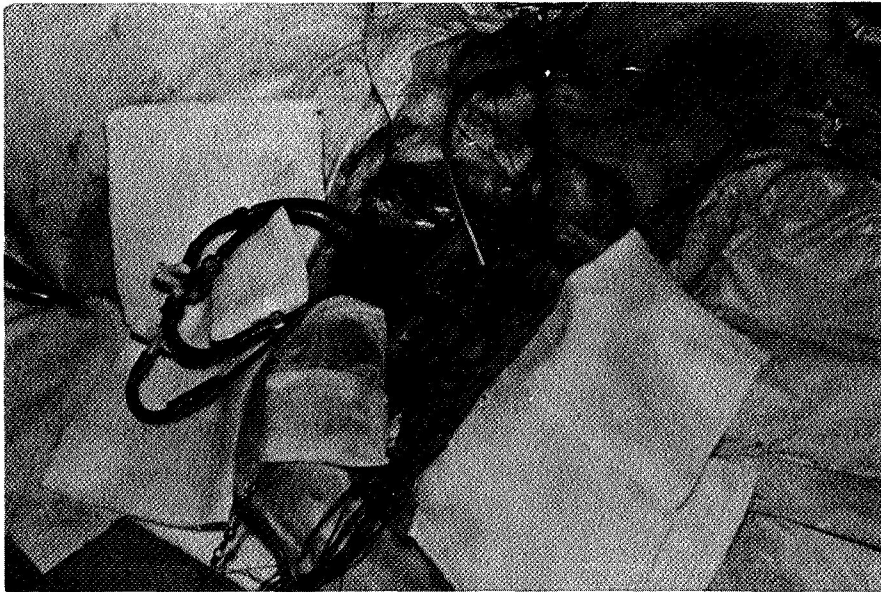


Fig. 2. Photograph of complete catheter circuit in place with isolated gracilis muscle in situ.

positioned and the animal was allowed to remain undisturbed to recover from the catheterization and stabilize for approximately 30 minutes. The catheters were wrapped with moist gauze and a servo-controlled lamp placed over them to insure a constant temperature of the perfusing blood.

4. Plethysmograph design. A clear plastic chamber was designed and fabricated (cf. Appendix A) into which the previously isolated gracilis muscle and vasculature were subsequently transferred and quickly reconnected to the femoral arterial and venous supply catheters. The muscle was anchored in the chamber by attaching the origin tendon to a fixed stainless steel clamp and the insertion tendon to an extendable clamp. Thus by extending the clamp, the muscle could be restretched to its in situ length. Series 300, cannulating-type, 1/16" I.D., electromagnetic blood flow probes with pressure ports were permanently mounted to the wall of the chamber to provide a non-restrictive, fluid-tight seal through which the tissue perfusion was re-established. The chamber contained a thermister probe which, when connected through a telethermometer (servo-controlled) to a 250 watt infrared lamp, maintained the temperature at 37.5°C.

The chamber top was bolted to the assembly with a 1/16 inch rubber gasket, generously lubricated with vacuum grease

which served as a pressure seal. The top of the chamber contained two female luer fittings which were used for filling the chamber and monitoring pressure. The entire plethysmographic chamber was positioned in a cradle in such a manner that the mean height was at the level of the animal's right atrium.

Figure 3 shows the plethysmograph and accessory equipment, with the muscle and catheters in place.

The pressure monitoring system consisted of wide bore glass tubing which connected the output luer fitting to a constant pressure reservoir. The glass tubing formed a continuous oil pathway from the chamber to the reservoir. The total volume of the reservoir was approximately  $100 \text{ cm}^3$ , with the air-phase normally being  $60 - 70 \text{ cm}^3$ . It was established that a  $2.0 \text{ cm}^3$  change in the volume of the plethysmograph resulted in a 1 - 2 mm change in the height of the oil column in the reservoir.

Consequently, hydrostatic back-pressure on the muscle preparation was minimized during fluctuations in chamber volume. For a more detailed description of the plethysmograph design, see Appendix A.

The air phase of the reservoir was continuous with the dome of a Statham P23-BB pressure transducer. This transducer recorded the pressure analog of volume change in the muscle. A

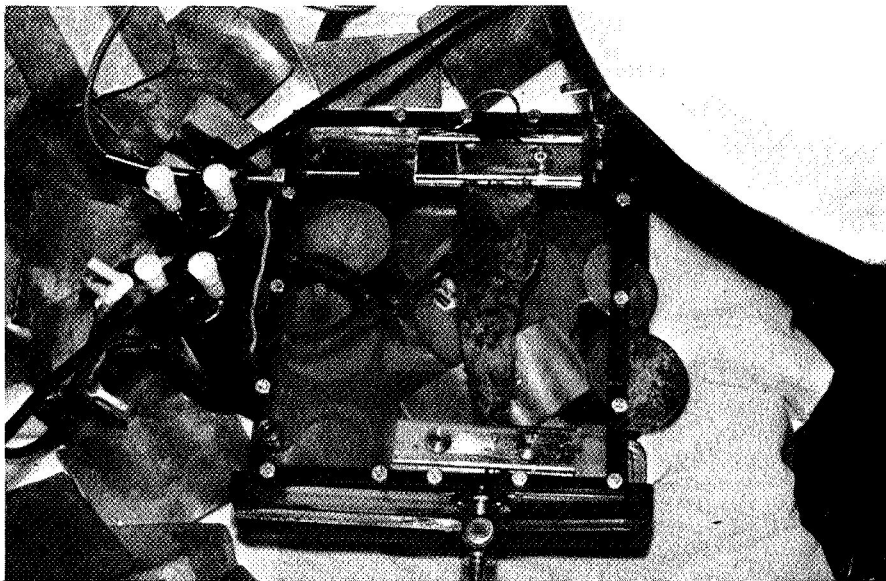


Fig. 3. Photograph of gracilis muscle being perfused through the catheter-flow probe circuit secured in the plethysmographic chamber. Note thermally isolated constant pressure reservoir attached to chamber in upper right corner.

small ceramic disc thermister was suspended freely in the air phase for continuous measurement of chamber temperature. The pressure transducer and constant pressure reservoir system were thermally isolated from the influence of the heat lamp by loosely wrapped, alternating layers of aluminum foil, cotton and nylon mesh, all of which was covered with styrofoam.

After the muscle was clamped in place and flow re-established, the plethysmograph was filled with a measured volume of mineral oil (specific gravity 0.85) pre-heated to  $37.5^{\circ}\text{C}$  in a water bath. Mineral oil was employed as the chamber fluid because of 1) its low electrical conductance, 2) its relative chemical inertness, and 3) to prevent the introduction of an osmotic variable to the changes in total tissue volume. The difference between the volume of oil required to fill the chamber containing only the fittings and catheters and that volume necessary to fill the chamber with the muscle in place represented the initial total volume of the muscle. In most instances, the pressure analog of this volume stabilized within 2 - 20 minutes after the chamber was sealed. This stabilized pressure was considered representative of the isovolumetric state of the muscle preparation. The preparation was routinely allowed to stabilize in the chamber for approximately 30 minutes before experimental manipulation of the

vascular bed was attempted.

A persistent problem throughout the study was the small amount of generalized oozing of heparinized blood from the fascial covering of the muscle surface. Several experiments were devoted to solving this problem with the final solution being 1) careful ligation of all potential ooze sites with fine suture, 2) a topical application of protamine sulfate to the muscle surface, and 3) in some experiments, an additional application of a 10% solution of  $\text{FeCl}_3$  to oozing sites. Particular care was taken to insure that the  $\text{FeCl}_3$  did not contact the vessels, nerve root, or any exposed muscle fibers. As a further means of reducing the effects of uncontrollable fluid accumulation, the chamber floor was constructed so as to trap and drain away any exudate through an orifice in the floor.

At the completion of each experiment all catheters, ligature material, and excess mineral oil were removed and the muscle weighed to the nearest 0.1 gram.

#### B. Technical Measurements (Data Acquisition)

Circulatory parameters recorded in the present investigation included gracilis arterial pressure (GAP), gracilis vein pressure (GVP), plethysmographic pressure calibrated in terms of volume ( $\Delta Q$ ), and gracilis vein outflow (GVF) (Fig. 4). In

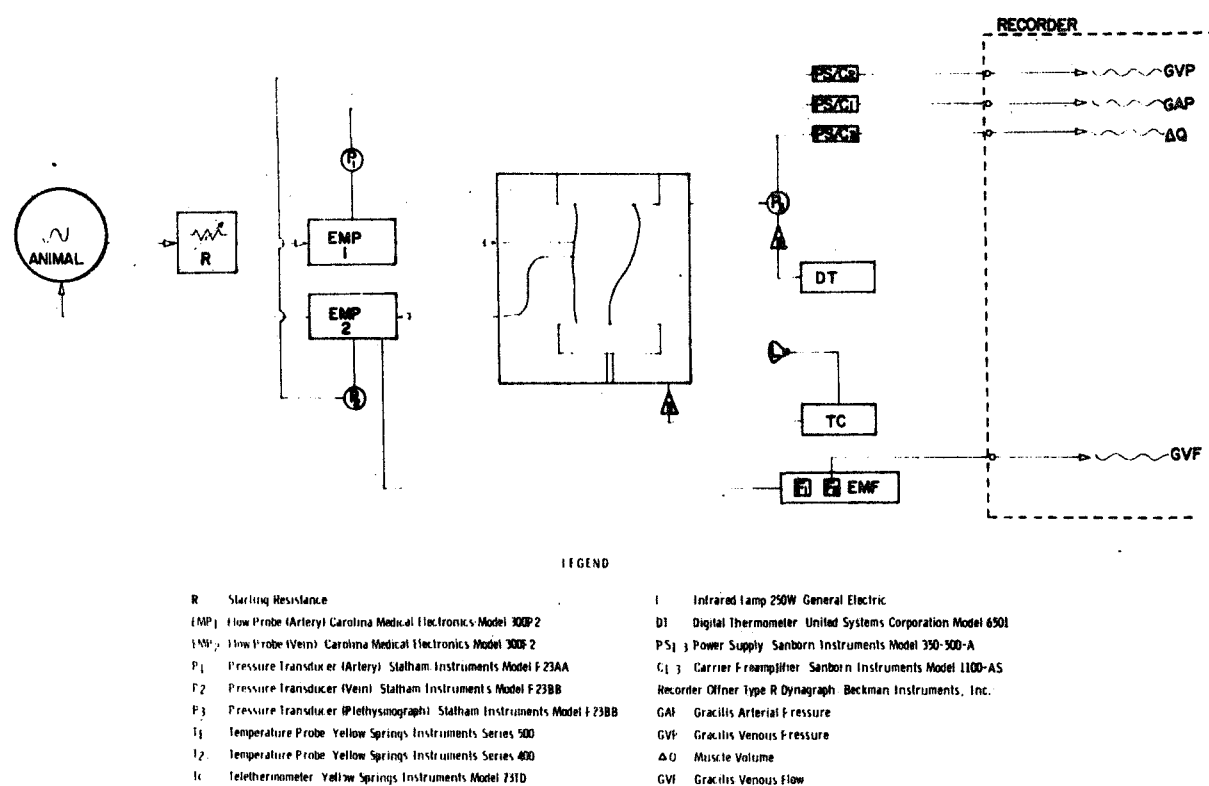


Fig. 4. Schematic diagram of instrumentation employed for measurement of vascular parameters.

addition, intravascular volume changes ( $\Delta IVQ$ ) were monitored by the activity of  $^{51}\text{Cr}$ -labeled red blood cells in the vascular space. Another parameter of interest was extravascular volume ( $\Delta EVQ$ ) which was derived as the numerical different between  $\Delta Q$  and  $\Delta IVQ$ .

1. Pressure-flow recording. Gracilis arterial pressure was recorded by a fluid-filled pressure transducer (Statham P23-AA) affixed to the downstream pressure tap of the arterial blood flow probe, and gracilis vein pressure was recorded by a similar transducer (P23-BB) attached to the upstream pressure tap of the venous blood flow probe. This placement of the transducers allowed an accurate calculation of perfusion pressure (gracilis arterial pressure minus gracilis venous pressure, noted  $\Delta P$ ) across the vascular bed. Measured pressure drops across the catheter circuit were insignificant at flows ranging from zero to approximately two times the largest flow registered in the course of the experiments, and accordingly, catheter resistance was considered to be negligible.

The output signals from the pressure transducers were conditioned through carrier amplifiers and fed into respective pre-amplifier stages of an 8-channel recorder for scaling and subsequent registration on a strip chart.



The two pressure recordings were referred to a common zero reference plane at the level of the right atrium (32). Calibration of these pressures, using a mercury manometer, was made routinely, both prior to and following each animal experiment; and periodic checks of baseline stability were made during the course of each experiment.

Plethysmographic pressure, recorded as an analog of muscle volume change, was sensed by an air-filled pressure transducer (P23-BB) attached to the constant pressure reservoir of the chamber. The output signal was conditioned in a manner similar to that of the circulatory pressures and was registered on the chart as  $\Delta Q$  per unit time. The plethysmograph was calibrated after the temperature had stabilized at  $37.5^{\circ}\text{C}$  with a non-viable muscle in place by injecting into and withdrawing known volumes of mineral oil and recording the resultant pressure changes. The dynamic response of the system is described in Appendix A.

Gracilis vein blood flow was monitored continuously by a highly sensitive, cannulating-type, 1/16 inch, electromagnetic flow probe and electromagnetic flowmeter whose output was registered on the recorded as blood flow in  $\text{cm}^3/\text{minute}$ . Since the EMP series 300 flow probes have been proven insensitive

to RBC concentration (6) the calibration of the flow monitoring system was based on the volume of saline solution collected per unit time. The accuracy of this specially designed flow probe was determined to be  $0.1 \text{ cm}^3/\text{minute}$  over a flow range of zero to  $20 \text{ cm}^3/\text{minute}$ . Although a similar probe was employed on the arterial side of the vascular supply to the muscle, this probe served only as a non-restrictive seal for the gracilis artery and served also as a manifold for measurement of arterial inflow pressure.

2. Compartmental fluid volume recording. Intravascular volume was recorded continuously by monitoring the activity of radio-labeled red blood cells ( $^{51}\text{Cr}$ ; Rachromate-51, Abbott) in the vascular space. Sixty cubic centimeters of venous blood were withdrawn from the animal and incubated with 1.5 millicuries of Rachromate-51 and 4.0 ml of ACD solution (Cenolate, Abbott) in a silicone-coated tagging vial for a period of 90 minutes (10). On completion of the incubation, 50 mg of ascorbic acid were introduced to reduce any unbound  $^{51}\text{Cr}$ , and the contents of the vial were centrifuged at 1800 rpm for five minutes. The supernatant fluid was discarded and the formed elements were resuspended in an equivalent volume of chilled isotonic saline. The washing was repeated a second time,

which, according to Albert (2), decreases the amount of unbound  $^{51}\text{Cr}$  to less than 0.3 per cent. The final volume of washed cells was resuspended in isotonic saline and injected slowly into the animal through the left femoral venous catheter. Extreme care was taken to insure that the total injected amount was flushed through the catheter and entered the animal's circulation.

The activity of the circulating radio-labeled red blood cells was monitored continuously by a shielded scintillation detector placed over the center of the muscle. The belly of the gracilis muscle fell within the diameter of the NaI crystal; and the entire muscle was within the solid angle of the detector assembly. The complete specifications of the detector assembly, including geometric considerations, are found in Appendix B.

The output of the photomultiplier tube within the detector assembly was amplified and introduced into a pulse height analyzer which discriminated 50 Kev above and below the energy peak of  $^{51}\text{Cr}$ . The output of the pulse height analyzer was divided electronically and fed simultaneously into a scaler for totalization of accumulated counts and into a linear ratemeter for continuous registration of count rate. The scaler was controlled by a pre-set timer to read the total number of counts over a pre-set time interval. The ratemeter output was

registered by a potentiometric recorder as count rate in temporal sequence with the other circulatory parameters. A two-second ratemeter time constant was employed routinely in all experiments.

At the conclusion of all experiments involving radioactivity, the size of the intravascular space was determined in the following manner. The arterial inflow and venous outflow catheters were occluded simultaneously and systemic arterial and venous blood samples withdrawn. The gracilis vascular space was then washed out by a slow injection of isotonic saline, and the venous effluent collected as described by Polosa and Hamilton (36). The infusion pressure was measured continuously during the washout to insure that the vasculature was not subjected to excessive pressures. The washout was terminated when both the radioactivity in the muscle was stable and no hemoglobin was visible in the venous effluent. The collected washout volumes were approximately 40 cm<sup>3</sup> in the preparations studied.

The calculations employed in determining the intravascular volume based on the washout are described in detail in Appendix C, part 2. Concisely, the intravascular volume (IVQ) was obtained from the ratio of activity in the saline washout to the specific activity of the perfusing blood. The temporal values of

count rate recorded throughout the experiment were corrected for background activity and scaled to reflect the size of the intravascular fluid compartment at the time of washout. Arterial and venous blood samples were collected periodically throughout the course of all experiments to insure that the blood radioactivity was constant. In none of the experiments reported was there any detectable change in blood activity.

Changes in extravascular volume (EVQ) were calculated as the numerical difference between the changes in muscle and intravascular volumes. No direct attempt was made to measure the size of the EVQ with an independent method in the present study.

### C. Protocol and Data Analysis

A typical experimental protocol in the second phase of the study consisted of acquiring a minimum of three separate pressure-flow curves. This was accomplished by reducing the gracilis arterial pressure in a stepwise manner by occluding a segment of rubber tubing in the inflow catheter circuit. Each pressure level was maintained until all parameters had stabilized. Steady-state values normally occurred within 30 seconds following each pressure decrement and a new pressure level was selected 60 seconds after equilibrium. Of the ten animals prepared for the second phase of the investigation, only six

complete experiments are reported. The remainder of experiments were discarded because of technical difficulties which prevented the registration of complete volume response data. The completed experiments in the second phase of the study (6 animals; 20 separate pressure-flow curves) yielded pressure, flow, and muscle volume data. Two of these animals (5 separate pressure-flow curves) also provided intravascular and extravascular volume data.

The recorded changes in the four experiments not selected for analysis were consistent with the responses recorded in those animals that were chosen.

## RESULTS

The changes in muscle volume ( $\Delta Q$ ) recorded in temporal sequence with gracilis vein flow (GVF) during stepwise reduction of perfusion pressure (gracilis arterial pressure minus gracilis venous pressure, noted  $\Delta P$ ), from 20 experiments in six animals are presented. In one series (Fig. 5), blood flow is expressed in cubic centimeters per minute ( $\text{cm}^3/\text{min}$ ) and perfusion pressure in millimeters of mercury (mm Hg). Conductance, defined as the ratio of GVF in  $\text{cm}^3/\text{min}$  to  $\Delta P$  in mm Hg, noted as URP (21), is expressed as per cent of control with 100% being that conductance observed at a  $\Delta P$  of 90 mm Hg.

In five of the twenty observations (2 animals) the sequential changes in intravascular volume ( $\Delta IVQ$ ) were determined simultaneously with the above mentioned parameters (see Fig. 8). In these five experiments, the relative changes in extravascular volume ( $\Delta EVQ$ ) were recorded as the numerical difference between  $\Delta Q$  and  $\Delta IVQ$ . In this series all blood flow and volume change data were normalized to 100 grams wet weight of muscle tissue and to a  $\Delta P$  of 140 mm Hg.

In the normalization procedures, zero on the volume

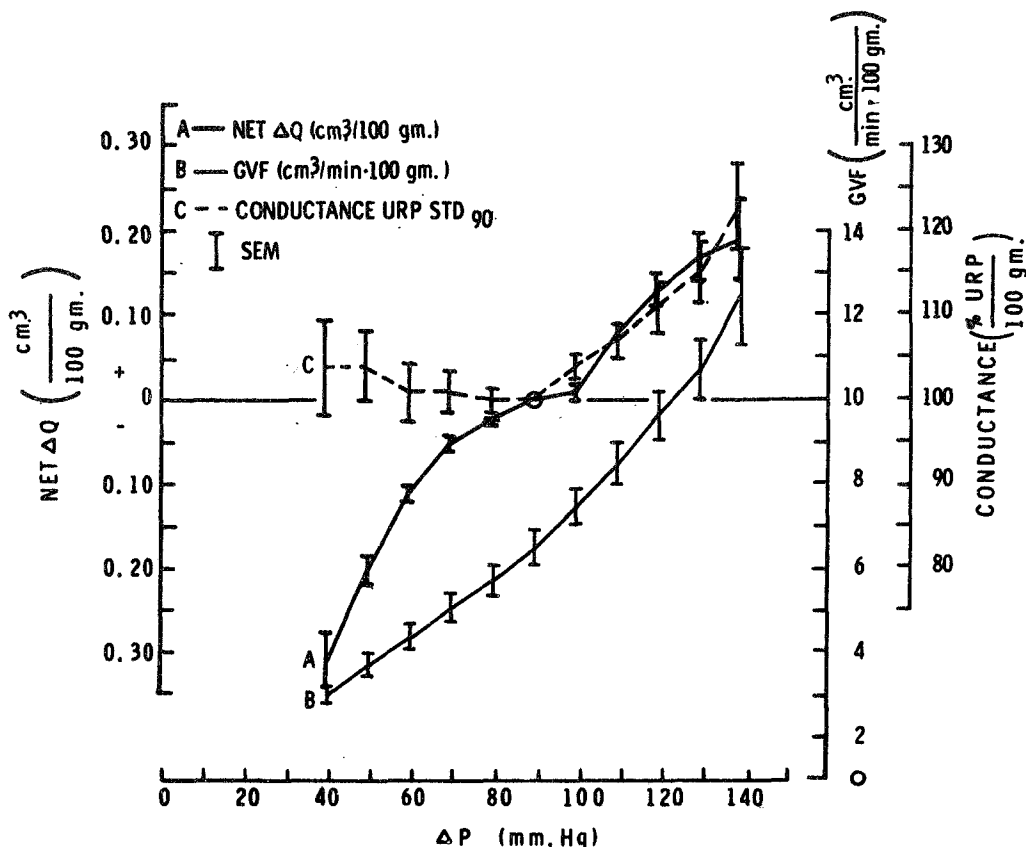


Fig. 5. Comparison of the response of total muscle volume, gracilis vein blood flow, and vascular conductance to stepwise reduction of perfusion pressure. The flows per 100 gm for each perfusion pressure were averaged and are plotted (B) as the mean flow at each level of perfusion pressure as:  $\overline{GVF}_{exp} = \frac{\sum GVF}{\frac{100 \text{ gm}}{N}}$ .

The mean conductance at each level of perfusion pressure was computed as the mean flow/100 gm  $\div$  the perfusion pressure as:  $\overline{URP}_{exp} = \frac{\overline{GVF}_{exp}}{\text{mmHg}_{exp}}$ .

The data in the plot (C) are normalized to 90 mm Hg  $\Delta P$  by dividing the conductance at each level of perfusion pressure by that at 90 mm Hg as:

$$\% \text{ URP}_{90} = \frac{\overline{URP}_{exp}}{\overline{URP}_{90}} \times 100$$

The plot of the change in total muscle volume (A) was computed as follows: the position of the recording line when the muscle was perfused at  $\Delta P = 90$  mm Hg was defined as zero  $\Delta Q$ . The deflection of the recording from zero was expressed as change of volume using the calibration factor as obtained from figures 12 and 13. This procedure was employed to facilitate comparison with data obtained by other investigators (7, 17, 44, 45). The volume changes were referenced to 100 gm of muscle weight by dividing the recorded volume change by the actual weight of the muscle and multiplying by 100.



ordinate represents the isovolumetric state of the preparation registered at resting perfusion pressure. Gains in volume by the preparation are indicated by ordinate values greater than zero and losses by values less than zero. The numerical values of the ordinate represent cumulative volume changes ( $\text{cm}^3$ ) from the normalized pressure value. Values of  $\Delta P$  greater than 140 mm Hg and less than 40 mm Hg were not observed frequently enough to have statistical significance and were therefore not reported. In the graphic representations of recorded data, population means were connected by a series of straight lines with vertical bars indicating  $\pm 1$  standard error of the mean (SEM).

The comparison of the changes in  $\Delta Q$  in temporal sequence with GVF and URP (Fig. 5, curves B and C) during stepwise reduction in  $\Delta P$  illustrates that muscle volume decreases paralleled vascular conductance at perfusion pressures in excess of 90 - 100 mm Hg. As perfusion pressure was reduced from 100 to 70 mm Hg, during which time the conductance was relatively constant, the rate of change of muscle volume per unit change of perfusion pressure was less than at higher pressures. In contrast, at  $\Delta P$  values below 70 mm Hg, the muscle volume decreased rapidly as perfusion pressure was lowered, during which time no corresponding change in conductance was

observed. Although inflection points are magnified by normalization at 90 mm Hg  $\Delta P$ , the overall temporal pattern of changes is evident. The averaged pressure-flow curve in these experiments does not show good autoregulation; however, the inflections in the response curve of muscle volume are aligned with perfusion pressure values in the "normal" autoregulatory range of skeletal muscle.

Figure 6 relates perfusion pressure to flow in the same 20 observations described in figure 5. However, in this representation, the pressure-flow responses were separated into two groups of ten observations, each based on the magnitude of flow at 90 mm Hg. Those plots having flow values greater than the mean ( $6.4 \text{ cm}^3/\text{min } 100 \text{ gm}$ ) of the 20 experiments were grouped and termed dilated curves and those with flows below the mean value were grouped and described as constricted curves. Both curves show autoregulation although neither curve shows a marked plateau segment of flow at pressure values between 90 and 60 mm Hg. However, the changes in cumulative muscle volumes recorded in the constricted bed show a plateau between 60 and 90 mm Hg (Fig. 7). This response, also present but to a lesser degree in the dilated bed, implies a stabilization of total muscle volume throughout the autoregulatory range of flow in

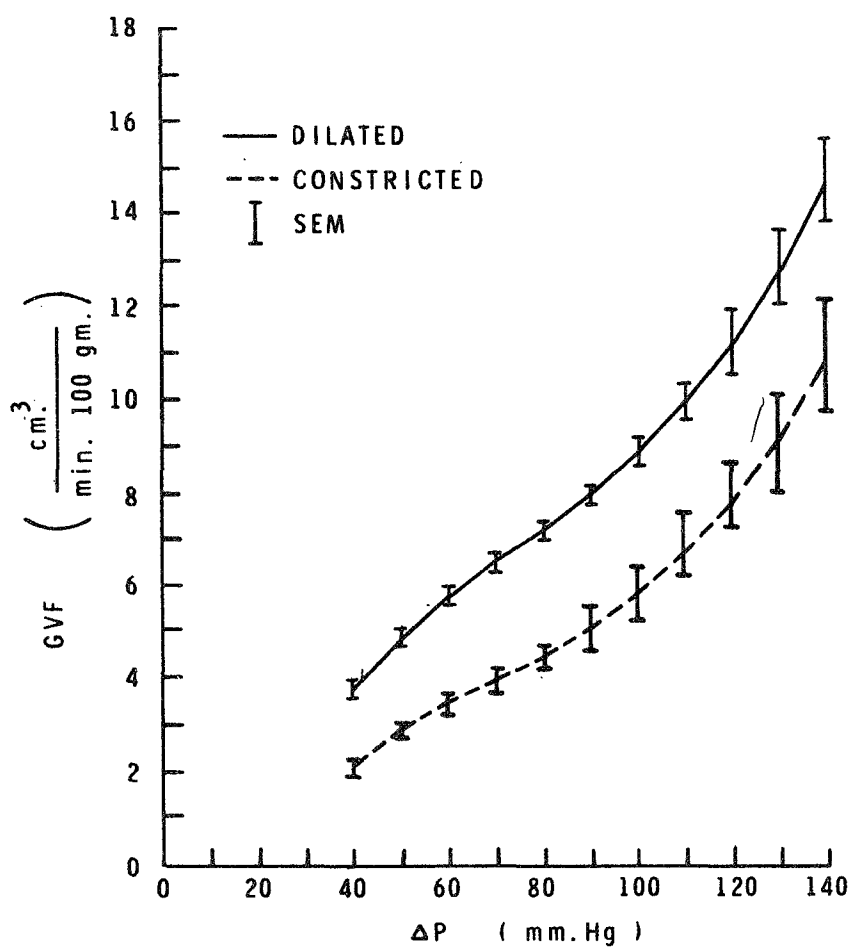


Fig. 6. The relationship between perfusion pressure and gracilis vein blood flow in two groups of experiments, separated on basis of mean value of blood flow recorded at 90 mm Hg perfusion pressure; individual  $N = 10$  observations.

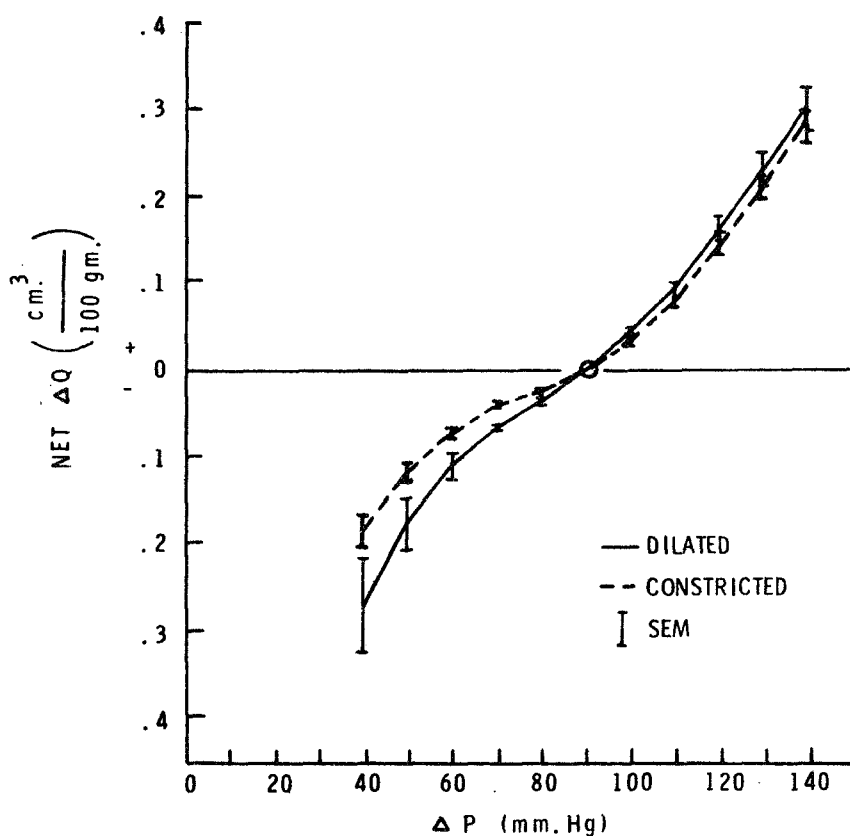


Fig. 7. Comparison of the muscle volume responses recorded by stepwise reduction of perfusion pressure in two groups of experiments separated on basis of mean value of blood flow at 90 mm Hg. Data normalized to perfusion pressure of 90 mm Hg; individual N = 10 observations.

skeletal muscle reported by Green (17) and Bond (7). The segments of the two plots recorded at perfusion pressures above 90 mm Hg are parallel and show no statistically significant difference.

The partition of fluid between intravascular and extravascular compartments is evident in the representations describing both total muscle and intravascular volume changes (Fig. 8). Three distinct, characteristic segments of the response of  $\Delta Q$  are evident in the plot. The initial decrease in the rate of volume change paralleled the decrease in conductance recorded over the range of  $\Delta P$  from 140 to 120 mm Hg. In the range of 120 to 70 mm Hg, the slope of the  $\Delta Q$  response was less, indicating a decrease in the volume change. At perfusion pressures less than 90 mm Hg, a pronounced fall in muscle volume was apparent. In this plot, as in figure 7, the decrement in muscle volume paralleled, in general, the recorded conductance changes through the pressure range of 140 to 70 mm Hg; however, below 70 mm Hg  $\Delta P$ , the marked fall in  $\Delta Q$  was not associated with any significant change in conductance. While the recorded response of intravascular volume also shows a characteristic pattern, the slope changes in this response are out of phase with those of both conductance and muscle volume at pressures from 140 to 70 mm Hg.

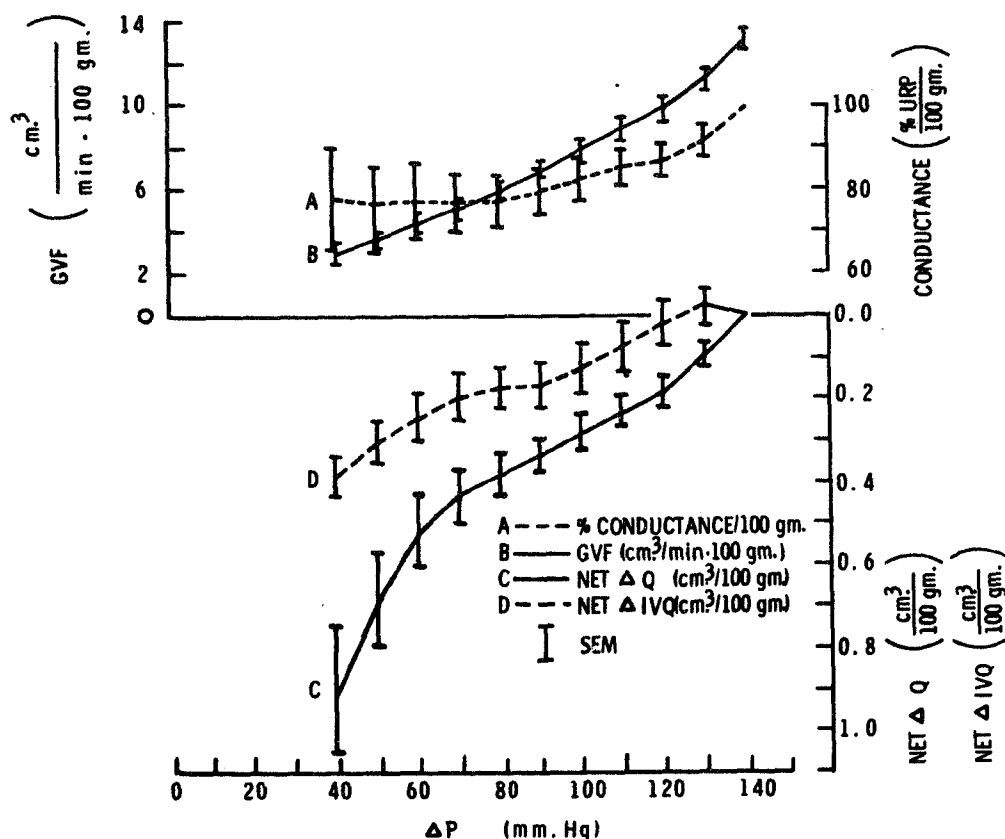


Fig. 8. The relationship between total muscle volume (C) and intravascular volume (D), recorded in the steady state after stepwise reduction in perfusion pressure. Conductance (A) is plotted as per cent of control with all recorded parameters normalized to a resting perfusion pressure of 140 mm Hg;  $N = 5$  observations. The change in muscle volume from 140 mm Hg  $\Delta P$  was computed in a manner similar to Figure 6; the change in intravascular volume was computed by subtracting the volume at a given pressure from that at 140 mm Hg and referenced to 100 gm of muscle weight.

Below 70 mm Hg  $\Delta P$ , a distinct inflection point corresponds to a rapid decrease in muscle volume. The IVQ diminished at a rate less than that of the muscle volume, indicating a continued reabsorption of fluid from the extravascular space at the lower perfusion pressures. The fact that the inflection points of both volume responses are reasonably well aligned with the autoregulatory range of  $\Delta P$  in skeletal muscle suggests that the volume decrements are in phase with the flow at the lower pressure values.

The significance of these changes is demonstrated in figure 9. In this figure the numerical difference between  $\Delta Q$  and  $\Delta IVQ$  (curve D) is plotted as the change in extravascular volume (curve C). The pressure-flow relationship (curve B) and conductance (curve A) is replotted in the upper graphs for convenience in relating the observed changes to vascular dynamics. The absolute value of the  $\Delta EVQ$  paralleled the recorded decrease in  $\Delta IVQ$  and the difference between the two was statistically insignificant at each pressure level. An approximately 1:1 relationship exists between the contributions of both the EVQ and IVQ as regards the redistribution of fluid volume from the organ studied to the systemic circulation as perfusion pressure is lowered.

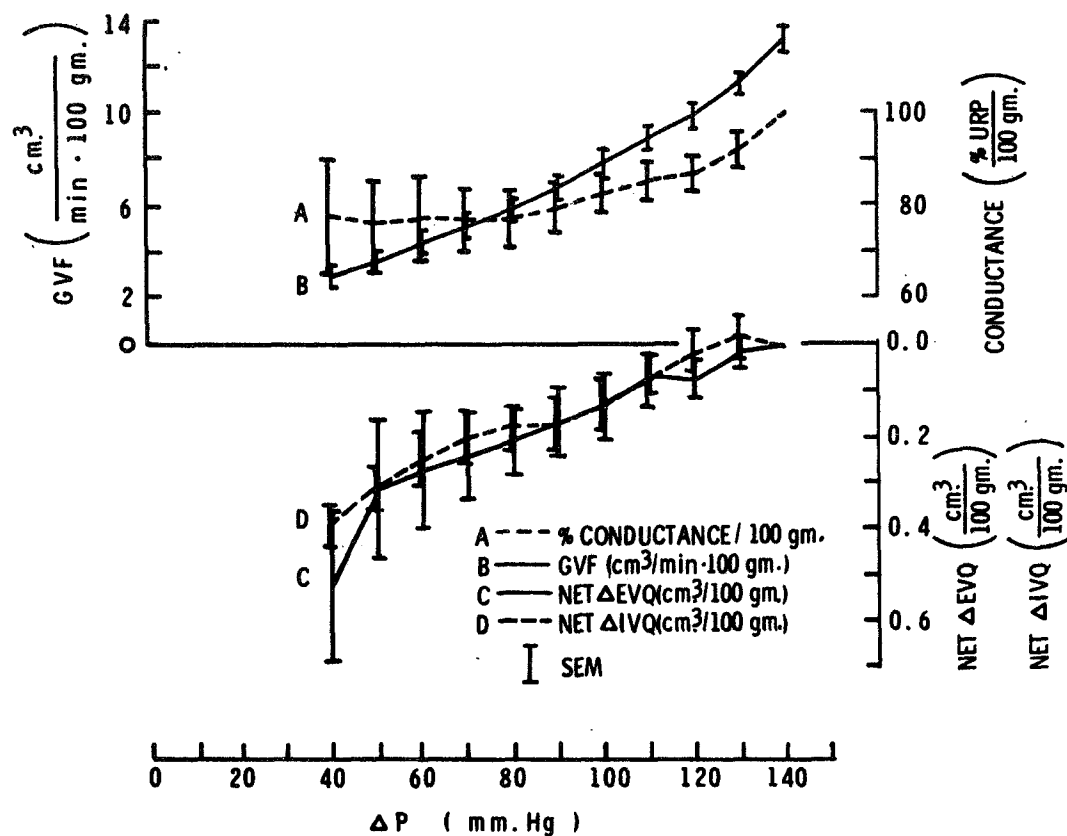


Fig. 9. Relationship between intravascular volume (D) and extravascular volume (C) during stepwise reduction in perfusion pressure. Data normalized to 140 mm Hg perfusion pressure (see Figure 8); N = 5 observations.



## DISCUSSION

### A. Introduction

Autoregulation of blood flow in peripheral vascular beds has been defined as the intrinsic tendency of an organ to maintain constant blood flow despite changes in arterial perfusion pressure (27). With the exception of kidney and skin, the blood flow to organs is closely regulated according to the metabolic demand of the organ. However, not only is the total organ flow of importance, but the partition of this flow between exchange and non-exchange pathways within the tissue also must be considered (37, 38). In vascular beds which show autoregulation of blood flow, the balance between the intrinsic and extrinsic modulator influences on the vasculature governs this partition of flow between the two networks.

The changes in the magnitude and direction of intercompartmental fluid shifts recorded in this investigation suggest that an intrinsic mechanism is operative in canine skeletal muscle that functions to stabilize intravascular and extravascular volumes. While autoregulation of blood flow, to some extent, serves the exchange function of the peripheral circulation, it also contributes to the deterioration of the systemic circulation through

the operation of the intrinsic mechanism. This latter mechanism serves to deplete the volume in the capacitance reservoir of the circulation by redistribution of extravascular fluid into the vascular space of the muscle at low perfusion pressures.

#### B. Critique of Biological Preparation

In the development of arguments and the presentation of data recorded in this investigation, evidence has been presented which indicates that a mechanism exists subsequent to the autoregulation of blood flow to the tissues whereby the steady state volume of fluid is also regulated by intrinsic modulator influences in the microcirculation. However, in the present study, pressure/flow responses and the related conductance plots do not show good autoregulation of blood flow. Classically, an increase in vascular conductance as the perfusion pressure is reduced through the autoregulatory range is commensurate with good autoregulation. Conductance values derived in this investigation show only a period of stable conductance, rather than a marked increase during the range of perfusion pressure usually described. There are several reasonable explanations for the lack of good autoregulation of blood flow in the muscle preparations studied. The presence of circulating vasodilator materials (52) such as histamine, epinephrine, adenosine triphosphate,  $\text{CO}_2$  or a

decreased  $O_2$  tension are among the many possible conditions in the vascular bed being studied that cannot be ruled out. Although during the course of the experiments, arterial and venous  $pO_2$ ,  $pCO_2$ , and pH measurements were within their respective normal physiological ranges, there can be no statement made as to their values at the specific effector sites in the microvasculature.

Even though a considerable amount of attention was given to maintaining the animal's physiological condition, the experiments were, at best, long and complicated, which necessitated prolonged anesthesia and tissue manipulation. Undoubtedly, levels of circulating dilator agents were present which may have led to a partial loss of vascular reactivity. One variable which may have been over-controlled was the environmental temperature of the gracilis muscle secured in the plethysmograph. Initially it was thought that an optimum ambient temperature for the muscle was  $37.5^\circ$  and considerable care was taken to insure that the muscle temperature did not vary more than  $0.5 - 1.0^\circ C$  from the temperature of the perfusing blood. However, it now seems possible that the normal temperature of a superficial muscle in the canine extremity may be lower than that of the perfusing blood. Although only intuitive at this point, it can be predicted from data presented by Green (20) that temperature

induced vasodilation was present in a large part of these experiments.

### C. Experimental Evidence

The data recorded in the present study identify a supportive function for autoregulation of blood flow related, in principle, to the metabolic (exchange) role of the microcirculation. This function is evident if one analyzes the relationship between the distribution of total organ or tissue flow and the intravascular pressures within the vasculature. In vascular beds which show autoregulation of blood flow, the precapillary pressure (13, 38) is maintained constant by the action of the arterioles and upstream resistance. Accordingly, intracapillary hydraulic pressure becomes a function of the perfusion pressure (precapillary minus postcapillary pressure) and the resistance across the capillary bed. By analogy, autoregulation of the total flow to the tissues, while serving in part to meet the metabolic demands of the organ, concomitantly provides a capillary hydraulic pressure which defines the balance between ultrafiltration and reabsorption of fluids (primarily water) and thereby stabilizes the intravascular volume. For example, if the precapillary vessels were tightly constricted, the capillary pressure would be lower than that when the same vessels are widely dilated. In the latter case, it is

possible that ultrafiltration of fluid from the intravascular space may occur during the decline to the lower perfusion pressures. Applying this reasoning to the analysis of pressure-flow curves recorded by Bond, Manley and Green (7), Stainsby and Renkin (44) and Stainsby (45), one can predict that capillary pressure may have been relatively high at lower perfusion pressures, i. e., declined less rapidly than systemic arterial pressure, in widely dilated vascular beds. At pressures below 60 - 70 mm Hg, capillary pressure presumably falls more rapidly than systemic arterial pressure as evidenced by a marked decrease of both total muscle and intravascular volumes. Conversely, in vascular beds which exhibit flow autoregulation to fluctuations in arterial pressure, intracapillary pressure is subsequently regulated in such a manner that the intravascular volume is also stabilized. The autoregulation of the equilibrium point of solvent exchange in skeletal muscle appears to be a well-founded possibility not only because the greatest blood volume and surface area are found in the venous capillaries and venules (48), but also because the permeability gradient across the capillary exchange segment favors solvent transport in the venous or venular capillary (31).

It is evident in figures 6 and 7 that a vascular bed which shows a tonic response of its precapillary resistance to changes

in perfusion pressure does in fact tend to regulate its intravascular volume by modulation of capillary hydraulic pressure. In figure 7, the total muscle volume response to stepwise reductions in perfusion pressure from 140 to 40 mm Hg is compared to the relative degree of vascular reactivity (tonic response) in two separable pressure/flow curves. It is apparent in this figure that total muscle volume is maintained more constant at lower perfusion pressures in the bed where autoregulation of blood flow was more pronounced. In other words, the lower curve in figure 6, described as active or constricted, shows better autoregulation than the upper curve; and consequently the total volume of the muscle tissue mass decreased less than that recorded from the dilated, or passive, response shown in the upper curve.

An explanation of this observation is evident when figures 8 and 9 are analyzed together. In figure 8, the intravascular volume (curve D) and total muscle volume (curve C) responses to stepwise reductions in perfusion pressure are plotted in sequence with the vascular dynamics (curves A, B). It is apparent that both the total muscle volume and the intravascular volume decreased non-uniformly across the range of pressure studied. However, of significance, is the fact that the intravascular volume was maintained essentially constant at perfusion pressures below 90 mm Hg. This

finding can be interpreted as a diminished reabsorption of extravascular fluid from the muscle tissue when perfusion pressures below 90 mm Hg were achieved. This interpretation is supported by figure 9 in which the net change in the extravascular volume (curve C) is related to the net change in intravascular volume (curve D) during stepwise decreases in perfusion pressure. Along the entirety of the volume response, there is no significant difference between the net change in intravascular and extravascular volume, suggesting that the extravascular volume (which is a portion of the total tissue volume of the muscle) was redistributed into the intravascular compartment throughout the range of pressures studied at a rate commensurate with stabilizing the intravascular compartmental space. It is evident in this figure (Fig. 9) that neither the extravascular nor total muscle volume response curves show a plateau segment, i. e., a period of relative isovolemia, throughout the entire range of perfusion pressure. In the intravascular volume response curve, however, a distinct plateau is evident which is significantly different from the total muscle volume response. These observations are consistent with a diminished rate of exchange of fluid from the extravascular to intravascular space at perfusion pressures between 90 and 50 mm Hg. Below 50 mm Hg perfusion pressure, the observed

redistribution of the extravascular fluid into the intravascular space would predictably lead to hemodilution of the systemic vascular reservoir. This interpretation is further supported by the fact that throughout the pressure range of 90 - 50 mm Hg, the rate of change of muscle volume is low compared to the other segments of the total response. Accordingly, it may be stated that this reabsorption of fluid implies a relatively stable intravascular volume throughout the autoregulatory range of blood flow in skeletal muscle. The mechanism by which this stabilization of the segmental volume of the vascular space occurs, suggests a modulatory regulation of capillary hydraulic pressure which favors the maintenance of a constant intravascular volume in the muscle bed.

Nonetheless, even in the absence of perfect autoregulation of blood flow in skeletal muscle as judged by the pressure/flow curves and derived values of vascular conductance, evidence is presented which indicates that the redistribution of compartmental fluid is controlled intrinsically in a manner similar to the maintenance of constant blood flow despite wide ranges of perfusion pressures. A suggested explanation for these findings is that an arteriolar dilation in the presence of a slight venular constriction would tend to maintain capillary hydraulic pressure at a value



which would favor a lesser decrease in the sizes of the intravascular and extravascular muscle fluid compartments as organ flow was diminished along its characteristic profile. Since vascular conductance did not autoregulate, as did the volume response, the slight increase in postcapillary resistance is a feasible explanation for the low flow values recorded as perfusion pressure was decreased from 90 - 60 mm Hg in the muscle vascular bed. Although speculative, the registration of an autoregulation curve does not exclude the possibility that the observed response is a summation of all vascular elements in the tissue, with the intrinsic responses of specific segmental sites in the microvasculature being masked by the complete event. The fact that volume transfer responses were recorded which imply an intrinsic regulation of the exchange elements in the circulation, in the absence of good autoregulation of blood flow, not only supports the concept of a masked response, but dramatizes the presence of an intrinsic, autoregulatory control of the preservation of the intravascular and extravascular volumes in the presence of a decreasing arterial pressure.

#### D. Conclusions

In conclusion, data has been presented which indicate that in the denervated canine gracilis muscle, an intrinsic mechanism is operative which is an integral part of the autoregulation of blood flow. The maintenance of constant flow to the exchange elements of the circulation despite wide fluctuations in arterial perfusion pressure provides, in addition, a regulation of capillary hydraulic pressure which defines the balance between ultrafiltration and reabsorption of fluid. This mechanism leads to an intrinsic autoregulation of solvent exchange and stable intravascular and extravascular volumes in the muscle bed. This intrinsic regulation of the exchange networks of skeletal muscle interferes with the desired systemic regulation by depleting the volume in the capacitance reservoir and reducing venous return. It may be predicted that the intrinsic response of the muscle to maintain its intravascular and extravascular volumes essentially constant during reductions in perfusion pressure is, in part, a significant contributor to the irreversible phase of hypotensive shock.

## APPENDIX A

### Design Considerations and Physical Specifications of the Plethysmographic Chamber

A plastic, rectangular-shaped chamber (Fig. 10) was fabricated of 3/8 inch Lucite<sup>R</sup> sections bonded with clear-drying epoxy cement. The top, also constructed of heavy gauge plastic, was secured to the chamber by 1/4 x 2 inch stainless steel ex-head machine screws spaced on 3/4 inch centers around the perimeter. A 1/6 inch fitted rubber gasket, generously lubricated with vacuum grease, served as a pressure seal between the chamber and top. The floor of the chamber was beveled horizontally to form a trough allowing fluid drainage when necessary. All openings into the chamber were of bonded, force-fit construction, and O-rings were employed for pressure seals. To reduce corrosion from salt solutions or other liquids, stainless steel, brass, or chrome-plated brass fittings were used throughout.

The chamber is shown in three views in figure 11. The overall dimensions of the chamber were kept as small as possible to minimize the total volume of the enclosure, and, thereby, enhance the sensitivity to small changes in tissue

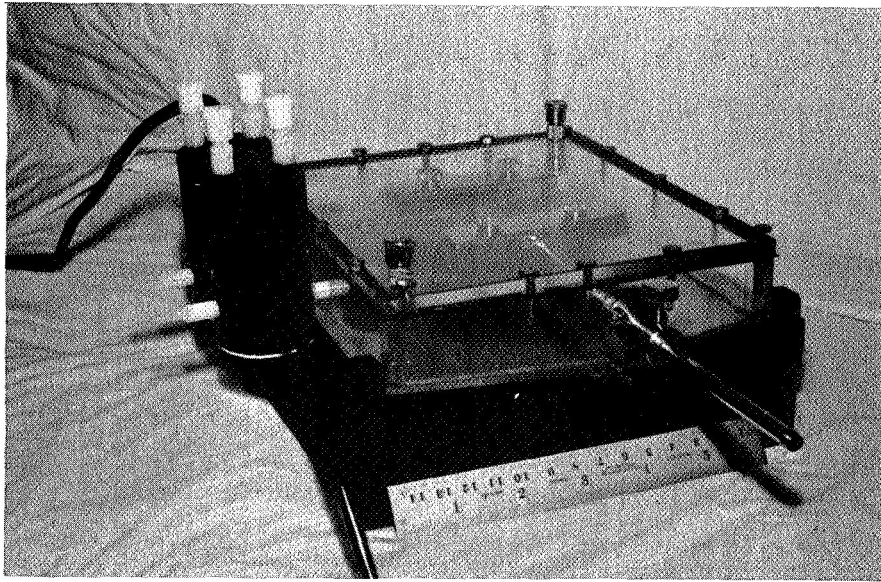
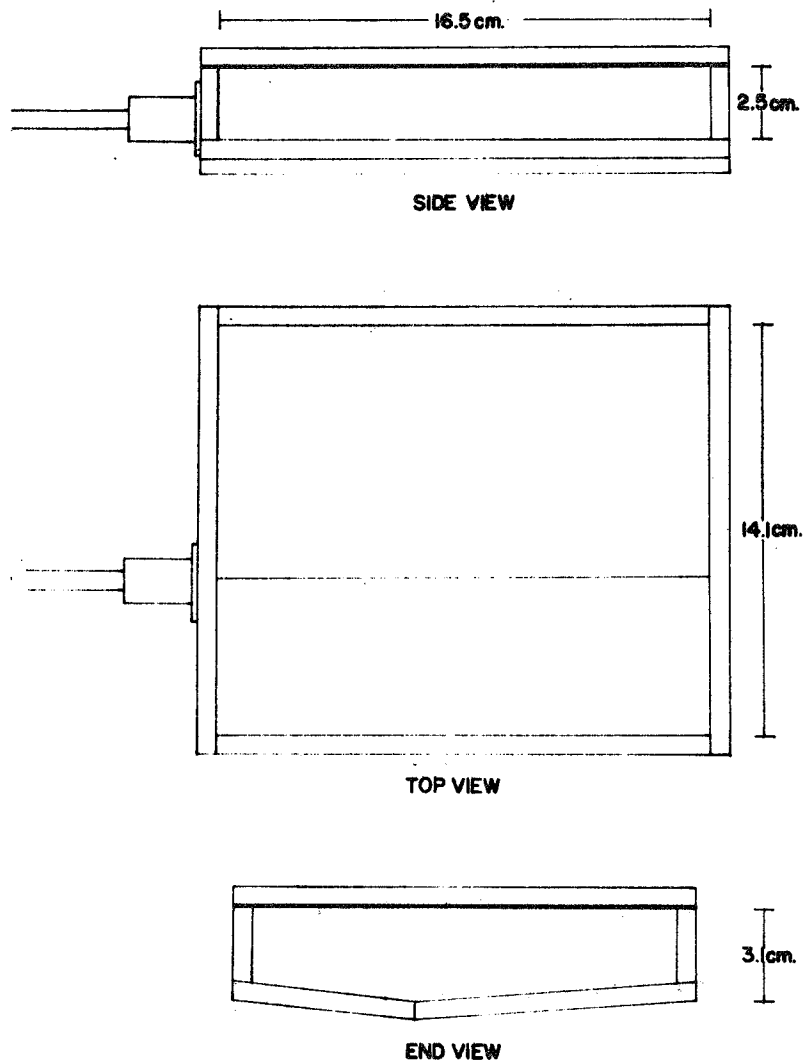


Fig. 10. Photograph of the plethysmographic chamber employed in this investigation. Note the two flowmeter probes to the left. The cannulae were used to obtain fluid "seals" between the chamber and animal.



MATERIAL: .65cm. LUCITE<sup>R</sup> CHAMBER; .05cm. RUBBER GASKET

Fig. 11. Critical dimensions of the plethysmograph chamber.  
Total volume, less hardware =  $649.0 \text{ cm}^3$ .

volume. At the same time, ample work space was available for securing the catheters between the animal and muscle. Prior to the final box construction, five experiments were conducted to determine the average dimensions of the gracilis muscle in the size animals selected for this study. From these results a clay model of the average gracilis muscle was constructed. This replica was the basis for the final dimensions of the plethysmograph which contained a volume of  $649.0 \text{ cm}^3$ , or approximately 15 times greater than the average sized muscle employed in the study.

The following design features distinguish this chamber from others employed in similar studies:

1. Hydrostatic pressure effects on the collapsible vascular channels were eliminated by using the rigid, non-collapsible flow ports of two cannulating-type, electromagnetic blood flow probes as fluid "seals" between the chamber and animal.

2. The origin tendon of the muscle was secured to a fixed stainless steel clamp and the insertion tendon to a variable clamp. Therefore, the resting, in situ length of the muscle could be restored in the chamber by extending the latter clamp. This capability minimized any effects of mechanical resistance on the supplying and draining vascular channels as a result of twisting

or severe bends in the vascular circuit.

3. The dimensions of the chamber allowed the entire muscle to be seen within the penumbral cone of a shielded scintillation detector placed directly over the muscle. Approximately 40 - 50% of the muscle tissue was covered by the circumference of the detector crystal (details of the detector assembly are found in Appendix B). This feature permitted an accurate registration of the isotope activity within the vascular space of the muscle while simultaneously reducing the contribution of background radioactivity to the total number of counts recorded.

The dynamic response of the plethysmographic chamber is shown in figures 12 and 13. These curves were recorded by injecting into and withdrawing from the chamber  $1.0 \text{ cm}^3$  of mineral oil with a nonviable muscle secured to the clamps. The temperature of the injected and withdrawn oil, as well as the contents of the chamber was maintained at  $37.5^\circ\text{C}$ . The amplified electrical output of the pressure transducer was scaled to trace a 20 mm deflection on the recorder chart for a volume change of  $1.0 \text{ cm}^3$  within the plethysmograph. This volume change produced an 8 mm Hg static pressure on the diaphragm of the transducer which was considered too great to have totally protected the muscle preparation from "back-loading". However,

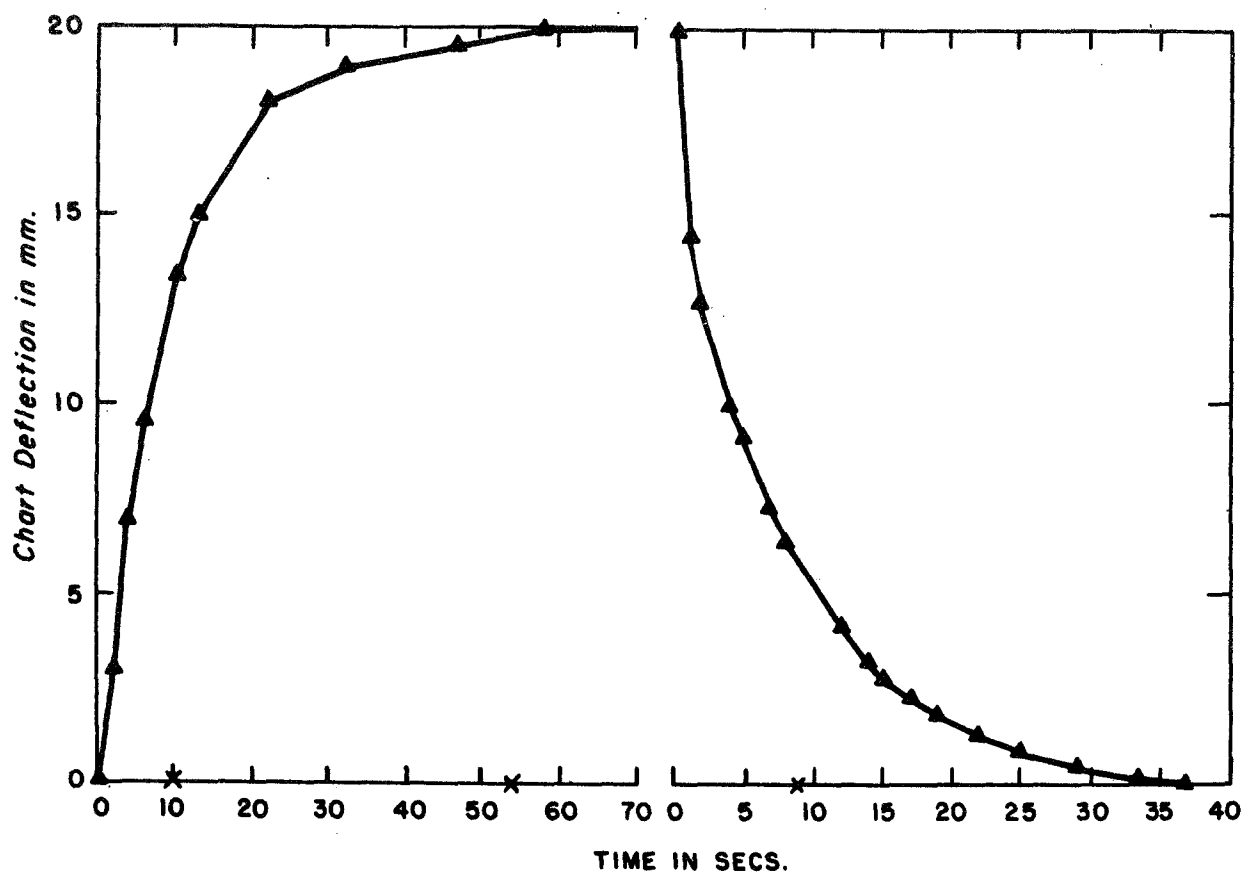


Fig. 12. The dynamic response of the plethysmograph recorded by rapid injection of  $1.0 \text{ cm}^3$  of mineral oil at  $37.5^\circ\text{C}$ . Time constants represented by stars on abscissa.

Fig. 13. The dynamic response of the plethysmograph recorded by rapid withdrawal of  $1.0 \text{ cm}^3$  of mineral oil at  $37.5^\circ\text{C}$ . Time constants represented by stars on abscissa.



in this study, all recorded vascular manipulations resulted in a volume decrease (analogous to withdrawal) rather than a volume gain. Consequently, the problem of creating a negative pressure effect on collapsible structures (principally the draining vein) must be considered. If one assumes that volume change (either gain or loss) produces a linear change in hydrostatic pressure, the maximum magnitude of induced pressure should not exceed approximately 6 mm Hg (negative) because maximum decrement of muscle volume was approximately  $0.75 - 0.80 \text{ cm}^3$ . Nevertheless, since collapsible structures were not completely protected from hydrostatic pressure effects, this is considered to be an error in the present study. The hysteresis of the system is apparent by comparing the time constants (starred values on abscissa) of the curves. In figure 12 (injection), 63% of the volume injected was registered in the initial 10 seconds, with 99% registered in 54 seconds. The withdrawal response (Fig. 13) shows 63% of the event complete in 8 seconds, and the total event complete in 37 seconds.

In two experiments conducted to check the sensitivity of the intravascular volume measurement (cf. Appendix B), known volumes of the animal's blood were injected incrementally into the arterial channel with the venous outflow occluded. When

compared to the injected amount, recorded volume changes in the chamber were insignificantly different between 0.1 and 0.5 cm<sup>3</sup>. These data are interpreted to mean that the ability of the chamber to accurately register changes in volume was not compromised by the relatively slow response registered by the rapid injection of 1.0 cm<sup>3</sup> oil into the plethysmograph. Provided that volume changes in the total muscle did not occur rapidly (as compared to the injection of oil into the chamber) the plethysmograph accurately reflected changes in muscle volume.

In an analysis undertaken following the conclusion of this series of experiments, it was found that the outflow circuit between the chamber and the constant pressure reservoir was imparting a mechanical resistance to flow of mineral oil between the two chambers which explains part of the reason for this slow response. This resistance prevented rapid pressure equalization between the two reservoirs (Figs. 12, 13) and, therefore, imparted a hydrostatic load on the contents of the plethysmograph.

It became evident early in the investigation that the Lucite walls of the chamber had a greater coefficient of expansion under thermal loading than the mineral oil. Consequently, when the servo-mechanism that controlled the internal temperature of the chamber energized the heat lamp, the chamber pressure would

decrease, an effect opposite to thermal expansion of the oil. A temporary solution to the problem was accomplished by moving the heat lamp a greater distance from the chamber such that the contents of the plethysmograph stabilized at a temperature of  $37.5^{\circ} \pm 0.5^{\circ}\text{C}$  without the necessity for automatic control.

Although this solution required manual monitoring of chamber temperature, it served its purpose as a temporary measure and was employed for all experiments in the present series.

## APPENDIX B

### Physical Characteristics of the System Employed

#### in the Measurement of Intravascular Volume

#### by $^{51}\text{Cr}$ -RBC Dilution

Figure 14 is a photograph of the scintillation detector in place over the plethysmograph containing the gracilis muscle. The principal component of this instrument is a 3.8 x 4.5 cm sodium iodide crystal (Thallium activated) which is enclosed in magnetic and thermal shields, and surrounded by one inch of lead. The crystal is mated with a photomultiplier tube within the confines of the lead, and the crystal-photomultiplier assembly is recessed 3.8 cm to achieve wide-bore, cylindrical collimation. A diagram of the total assembly, including other supportive electronics, is shown in figure 15. The total distance from the center of the suspended muscle to the face of the crystal is 5 cm.

Figures 16 and 17 are lateral and top views, respectively, of the shielded detector and plethysmographic assemblies showing the areas subtended by the penumbra, by the maximal solid angle, and by the crystal face as they relate to the muscle mass.

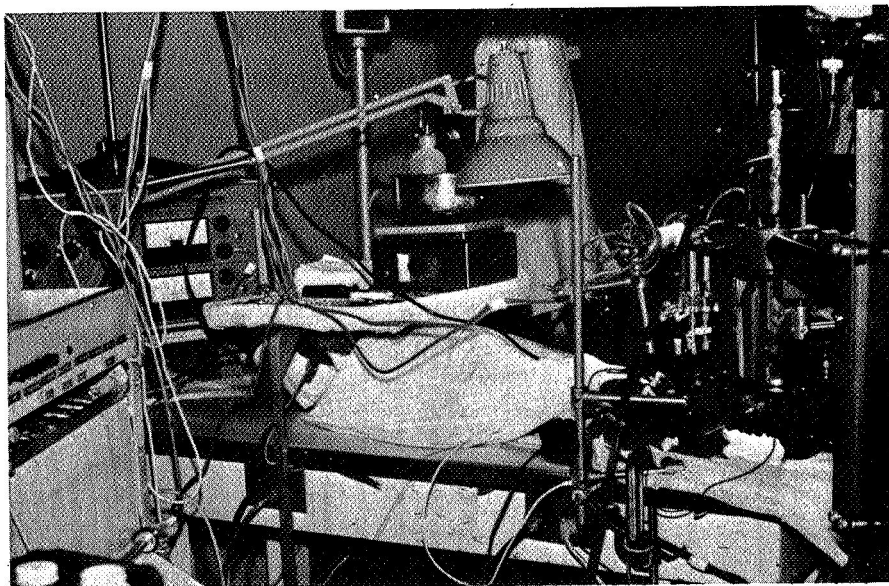


Fig. 14. Photograph of the shielded scintillation detector in place above plethysmograph during a typical experiment.

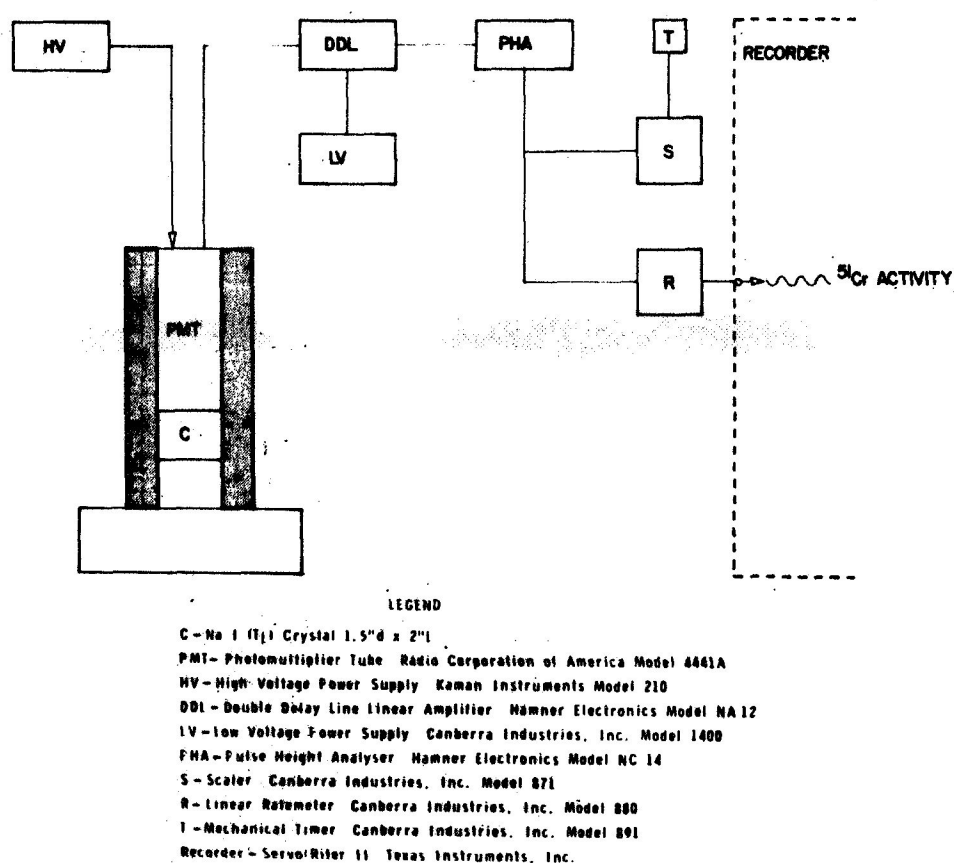


Fig. 15. Schematic diagram of instrumentation employed for detection and registration of the activity of  $^{51}\text{Cr}$  in the measurement of intra-vascular volume.

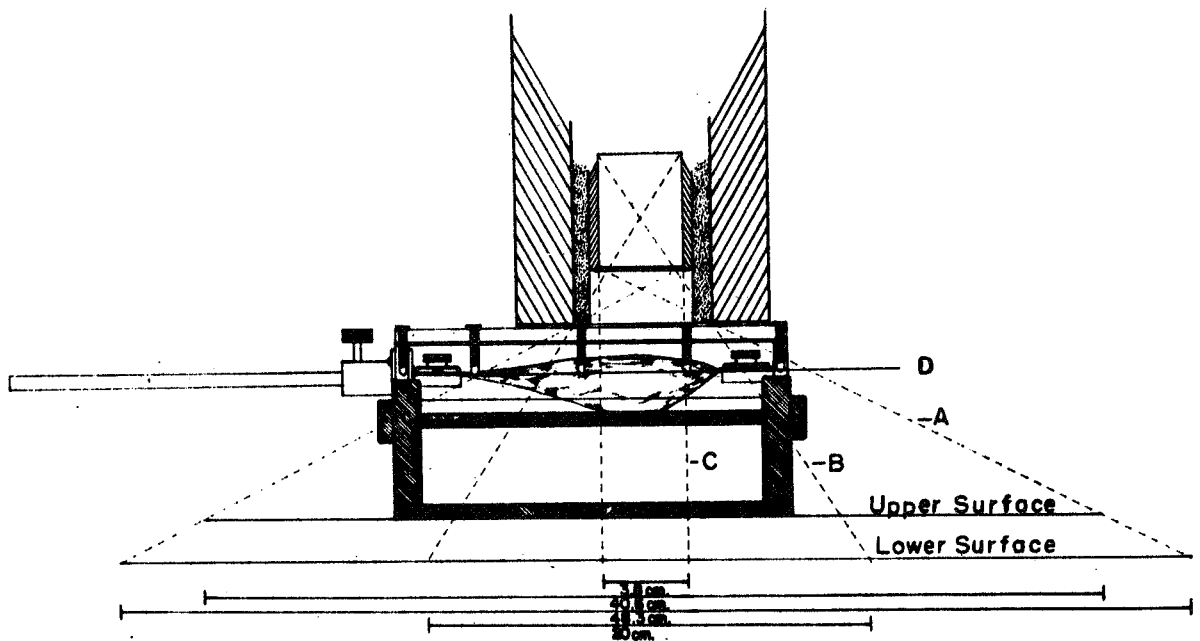


Fig. 16. Lateral view of scintillation detector assembly placed over plethysmograph describing the conical areas subtended by the penumbra (A), the total solid angle (B), and the crystal circumference (C) in relation to the mass of the gracilis muscle. Line D is the horizontal plane passing through the center of the muscle. Surfaces refer to top and bottom of surgical table.

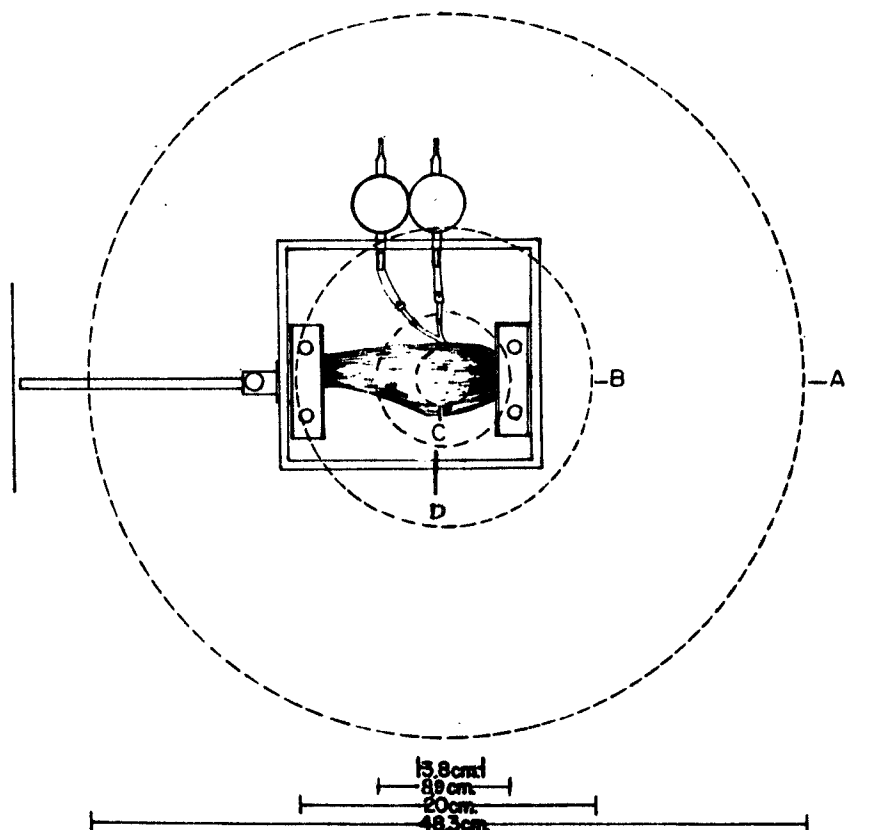


Fig. 17. Top view of scintillation detector assembly placed over plethysmograph describing the surface areas subtended by the penumbra (A), the total solid angle (B), and the crystal circumference (C) in relation to the mass of the gracilis muscle projected to lower surface of table. Circle D corresponds to the intersection of Line B with the horizontal plane of the muscle (Line D) in Figure 16.



It should be noted in these illustrations that although approximately only 40 - 50% of the muscle tissue falls within the circumference of the crystal, the entire muscle falls within the penumbra of the detector.

The plethysmograph and its contents were shielded from the animal and its surroundings by several segments of lead (1/4 inch; variable lengths and widths) placed between the hindquarters of the animal and the chamber cradle. A segment of lead sheet was placed vertically between the electromagnetic blood flow probes and the chamber wall to further shield the detector from the catheter circuit.

Simons (41) simplified the complex mathematical derivation for the loss in counting efficiency resulting from the movement of an object within the plane of a collimated scintillator (Fig. 18). By applying this relationship, one can determine the relative counting efficiency of the scintillator used in this study for any point shown in figures 16 and 17. In figure 19, a schematic representation of the catheter circuit employed in this study for perfusion of the gracilis muscle is superimposed on a plot of the total counts recorded from a point source moved along the course of the catheter circuit. This latter plot correlates with the theoretical relationship described by Simons

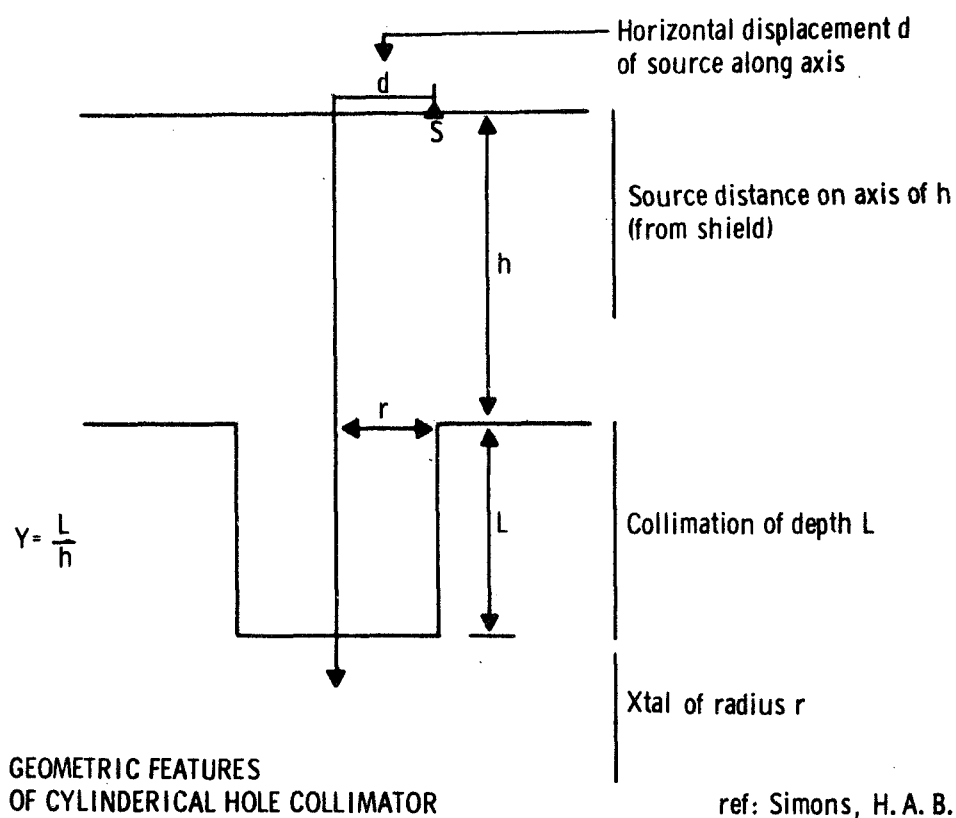


Fig. 18. The physical relationship between the object and scintillator assembly considered in the derivation of the loss of counting efficiency due to geometric considerations (reproduced from Simons [41]).

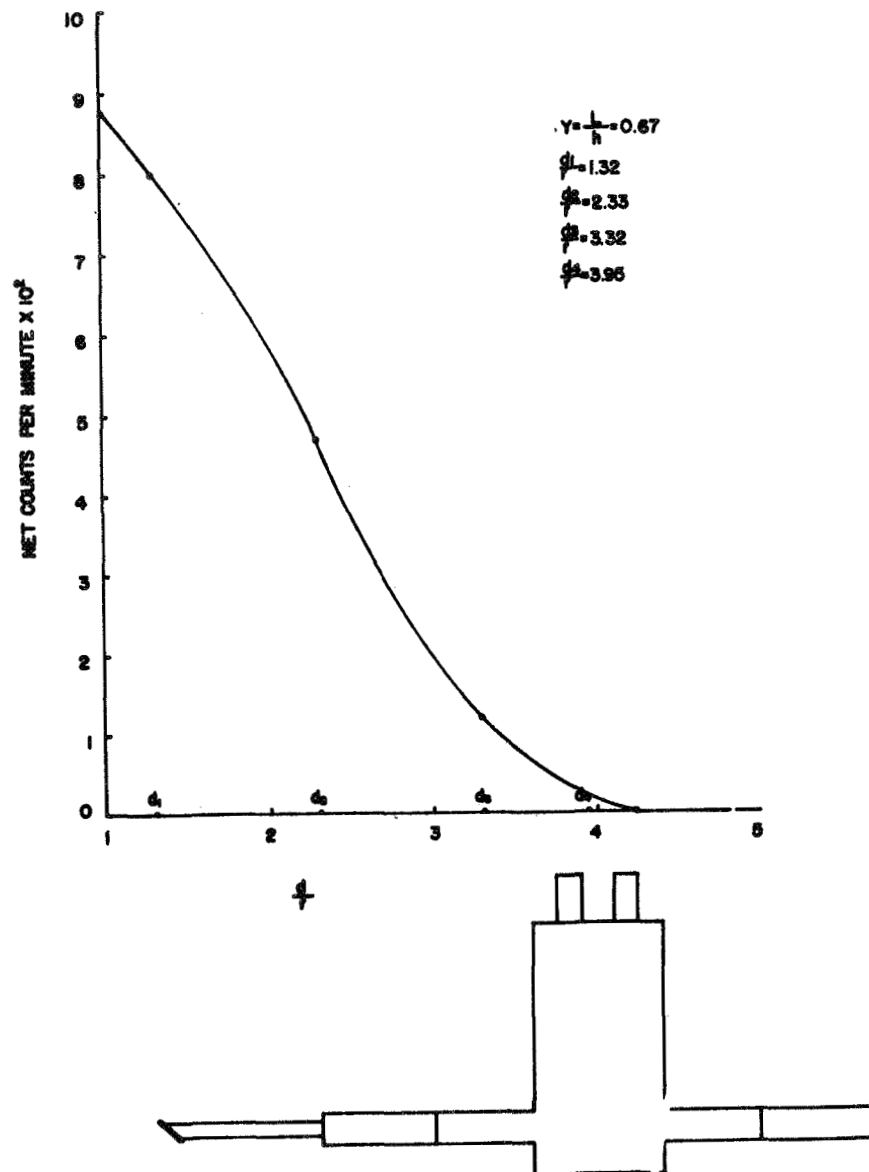


Fig. 19. Schematic representation of extracorporeal catheter circuit employed for muscle perfusion and the recorded activity of isotopic source as a function of distance from the center of the muscle. Values of  $d/r$  are spatially related to segments of catheter circuit in this diagram. Point  $d_2$  corresponds to the intersection of Line B with the horizontal plane (D) of the muscle in Figure 16, and with Circle D in Figure 17.

(41). The catheter circuit volume, relative to the total volume of both the muscle vascular bed and catheters, was determined in this manner (cf. Methods).

The recorded activity of the  $^{51}\text{Cr}$ -RBC in the intravascular space was scaled to reflect the size of the vascular volume at the time of washout. Two experiments were performed to check the sensitivity of the method to accurately measure changes in the segmental vascular volume. This was done by simultaneously occluding the inflow and outflow channels, followed by a stepwise injection of known volumes of the animal's blood containing radioactivity. The resultant changes in the  $^{51}\text{Cr}$  activity recorded by the detector were compared to the dilution factor determined by washout. It was noted that injected volumes (0.1 to 0.5  $\text{cm}^3$  in 0.1  $\text{cm}^3$  increments) were recorded by the detector in such a manner that no difference in injected and recorded volumes was apparent from 0.1 - 0.5  $\text{cm}^3$ .

## APPENDIX C

### Glossary and Calculation of Intravascular Volume

#### from Saline Washout

#### 1. Glossary

The abbreviations, symbols, and acronyms which appear in the text are defined below:

BCPM: Background counts per minute

CCQ: Catheter circuit volume

$^{51}\text{Cr}$ : Radioactive chromium

$\Delta\text{EVQ}$ : Change in extravascular volume, derived as the numerical difference between  $\Delta\text{Q}$  and  $\Delta\text{IVQ}$

$\Delta\text{IVQ}$ : Recorded change in intravascular volume of the gracilis muscle vascular bed

$\Delta\text{P}$ : Perfusion pressure; in this study, the difference between gracilis arterial pressure and gracilis venous pressure

$\Delta\text{Q}$ : Recorded change in total muscle tissue volume

GAP: Gracilis artery pressure

GCPM: Gross (total) counts per minute; recorded  $^{51}\text{Cr}$  activity

GVP: Gracilis vein (blood) flow

GVP: Gracilis vein pressure

KEV: One thousand electronvolts; energy unit of  $^{51}\text{Cr}$  activity

NCPM: Net counts per minute; derived as numerical difference between GCPM and BCPM

STD 90: Normalization of recorded data to  $\Delta P$  value of 90 mm Hg

STD 140: Normalization of recorded data to  $\Delta P$  value of 140 mm Hg

URP: Vascular conductance, defined as the ratio  $\text{GVF}/\Delta P$

WOQ: Washout volume

## 2. Calculation of Intravascular Volume from Saline Washout

(36, 46).

Samples ( $0.2 \text{ cm}^3$ ) of the arterial blood and venous washout effluent were prepared in triplicate and their activities ( $^{51}\text{Cr}$ ) were determined in an automatic gamma counting system (Auto-gamma - Packard Instruments, Model 3002). After the activities were averaged and corrected for background, the following calculations were performed:

Arterial sample:

$$\frac{\text{NCPM}_a}{(0.2 \text{ cm}^3)} \cdot \frac{5 (0.2 \text{ cm}^3)}{1.0 \text{ cm}^3} = \frac{\text{NCPM}_a}{\text{cm}^3}$$

Venous effluent sample:

$$\frac{\text{NCPM}_v}{(0.2 \text{ cm}^3)} \cdot \frac{5 (0.2 \text{ cm}^3)}{1.0 \text{ cm}^3} \cdot \text{WOQ} (\text{cm}^3) = \text{NCPM}_v$$

The following ratio of the activities describes the dilution space:

$$\frac{\text{NCPM}_v}{\frac{\text{NCPM}_a}{\text{cm}^3}} = \text{cm}^3$$

The derived value for the dilution space corresponds to the registered net activity of the muscle recorded with artery and vein clamped prior to the washout. The sequential changes in the size of the intravascular space were determined by scaling the recorded activities during the total experiment to reflect the size of the dilution space at the time of washout. This was accomplished by multiplying the continuously recorded values of the  $^{51}\text{Cr}$  activity (corrected for background activity) by the dilution (washout) factor. This factor was defined as the ratio of the measured size of the intravascular space to the net activity in the muscle determined at washout. The calculations performed were as follows:

$$\text{NCPM}_{\text{continuous}} \cdot \frac{\text{washed out IVQ (cm}^3\text{)}}{\text{NCPM washout}} = \text{cm}^3.$$

## BIBLIOGRAPHY

1. Åblad, B. and S. Mellander. Comparative effects of hydralazine, sodium nitrite and acetylcholine on resistance and capacitance blood vessels and capillary filtration in skeletal muscle in the cat. *Acta Physiol. Scand.*, 58: 307-319, 1963.
2. Albert, S. N. Blood Volume. Springfield, Ill.: Charles C. Thomas, 1963.
3. Baez, S. Bayliss response in the microcirculation. *Fed. Proc.*, 27: 1410-1414, 1968.
4. Baker, C. H., and L. J. O'Brien. Vascular volume changes in the dog forelimb. *Am. J. Physiol.*, 206: 1291-1298, 1964.
5. Barcroft, H., and O. G. Edholm. Effect of temperature on blood flow and deep temperature in human forearm. *J. Physiol.*, 102: 5-20, 1943.
6. Bond, R. F. "In vivo" method for calibrating electromagnetic flowmeter probes. *J. Appl. Physiol.*, 22: 358-361, 1967.
7. Bond, R. F., E. S. Manley, Jr., and H. D. Green. Cutaneous and skeletal muscle vascular responses to hemorrhage and irreversible shock. *Am. J. Physiol.*, 212: 488-497, 1967.
8. Brodie, T. G., and A. E. Russell. On the determination of the rate of blood-flow through an organ. *J. Physiol.*, 32: 47-49, 1905.
9. Cobbold, A., B. Folkow, I. Kjellmer, and S. Mellander. Nervous and local chemical control of pre-capillary sphincters in skeletal muscle as measured by changes in filtration coefficient. *Acta Physiol. Scand.*, 57: 180-192, 1963.



10. Cooper, M., and C. A. Owen. Labeling human erythrocytes with radiochromium. *J. Lab. Clin. Med.*, 47: 65-71, 1956.
11. Cutting, W. C. General anesthetics. In Handbook of Pharmacology. 3rd ed. New York: Appleton-Century-Crofts, 1967, pp. 478-479.
12. Folkow, B. Range of control of the cardiovascular system by the central nervous system. *Physiol. Rev.*, 40: 93-99, 1960.
13. Folkow, B., and B. Oberg. Autoregulation and basal tone in consecutive vascular sections of the skeletal muscle in reserpine-treated cats. *Acta Physiol. Scand.*, 53: 105-113, 1961.
14. Folkow, B. Autoregulation in muscle and skin. *Circ. Res.*, 14 and 15: Suppl. I, 19-24, 1964.
15. Gauer, O. H., and J. P. Henry. Circulatory basis of fluid volume control. *Physiol. Rev.*, 43: 423-481, 1963.
16. Gauer, O. H. Osmocontrol versus volume control. *Fed. Proc.*, 27: 1132-1136, 1968.
17. Green, H. D., R. N. Lewis, N. D. Nickerson, and A. L. Heller. Blood flow, peripheral resistance and vascular tonus, with observations on the relationship between blood flow and cutaneous temperature. *Am. J. Physiol.*, 41: 518-536, 1944.
18. Green, H. D. Circulatory System: Physical Principles. In Medical Physics. vol. 2. Otto Glasser (ed.). Chicago: The Year Book Publishers, Inc., 1950, p. 231.
19. Green, H. D., A. B. Denison, Jr., C. E. Rapela, and G. Lin. Use of indicator concentration curves in computation of mean rate of flow and volume of blood contained within a segment of the vascular system. *IRE Transactions Med. Electronics*, 6: 277-282, 1959.
20. Green, H. D., and J. H. Kepchar. Control of peripheral resistance in major systemic vascular beds. *Physiol. Rev.*, 39: 617-686, 1959.

21. Green, H. D., C. E. Rapela and M. C. Conrad. Resistance (conductance) and capacitance phenomena in terminal vascular beds. In Handbook of Physiology, Section 1, Circulation, vol. II, W. F. Hamilton and P. Dow (eds.). Washington, D. C.: American Physiological Society, 1963, pp. 585-615.
22. Gregersen, M. I. Some effects of reflex changes in blood pressure on plasma volume. Am. J. Physiol., 129: 369-370, 1940.
23. Gregersen, M. I., and R. A. Rawson. Blood volume. Physiol. Rev., 39: 307-342, 1959.
24. Guyton, A. C., A. W. Lindsey, and B. N. Kaufman. Effect of mean circulatory filling pressure and other peripheral circulatory factors on cardiac output. Am. J. Physiol., 180: 463-468, 1955.
25. Guyton, A. C. Circulatory Physiology: Cardiac Output and Its Regulation. Philadelphia: W. B. Saunders, 1963, pp. 315-322.
26. Himwich, H. E., and W. B. Castle. Studies on the metabolism of muscle. Am. J. Physiol., 83: 92-113, 1927.
27. Johnson, P. C. Review of previous studies and current theories of autoregulation. Circ. Res., 14 and 15: Suppl. I, 2-9, 1964.
28. Jones, R. D., and R. M. Berne. Local regulation of blood flow in skeletal muscle. Circ. Res., 15: 1-30, 1964.
29. Landis, E., and J. Hortenstine. Functional significance of venous blood pressure. Physiol. Rev., 30: 1-32, 1950.
30. Lawson, H., and W. S. Rehm. The effect of hemorrhage and replacement on the apparent volume of plasma and cells. Am. J. Physiol., 144: 199-205, 1945.
31. Majno, G., G. I. Schoefl, and G. E. Palade. Studies on inflammation. II. The site of action of histamine and serotonin on the vascular tree; a topographic study. J. Biophys. Biochem. Cytol., 11: 607-626, 1961.

32. Malindzak, G. S., Jr., C. E. Rapela and H. D. Green. Static method for simultaneous calibration of several pressure-transducer systems. *Med. Res. Eng.*, 7: 39-42, 1968.
33. Martin, E. G., J. Field, II, and V. E. Hall. A technique for obtaining and recording isometric contractions of mammalian skeletal muscles in situ. *Am. J. Physiol.*, 102: 476-480, 1932.
34. Mellander, S. Comparative studies on the adrenergic neurohormonal control of resistance and capacitance blood vessels in the cat. *Acta Physiol. Scand.*, 50: 3-86, 1960.
35. Oberg, B. Effects of cardiovascular reflexes on net capillary fluid transfer. *Acta Physiol. Scand.*, 62: 3-98, 1964.
36. Polosa, C., and W. F. Hamilton. Blood volume and intravascular hematocrit in different vascular beds. *Am. J. Physiol.*, 204: 903-909, 1963.
37. Renkin, E. M., and S. Rosell. The influence of sympathetic adrenergic vasoconstrictor nerves on transport of diffusible solutes from blood to tissues in skeletal muscle. *Acta Physiol. Scand.*, 54: 223-240, 1962.
38. Renkin, E. M. Normal regulation of tissue circulation. In Effects of Anesthetics on the Circulation. H. L. Price and P. J. Cohen (eds.). Springfield, Ill.: Charles C. Thomas, 1964, pp. 171-181.
39. Renkin, E. M. Neurogenic factors in microcirculatory low flow states. In Microcirculation as Related to Shock. D. Shepro and G. P. Fulton (eds.). New York: Academic Press, Inc., 1967, pp. 138-148.
40. Ross, J., C. Frahm, and E. Braunwald. Influence of carotid baroreceptors and vasoactive drugs on systemic vascular volume and venous distensibility. *Circ. Res.*, 9: 75-82, 1961.

41. Simons, H. A. B. A comparison between collimator theory, extended to allow for the effect of wall penetration, and experiment. In Medical Radioisotope Scanning. vol. I. Vienna: International Atomic Energy Agency , 1964, pp. 115-139.
42. Sjostrand, T. Blood Volume. In Handbook of Physiology, Section 2, Circulation, vol. I. W. F. Hamilton and P. Dow (eds.). Washington, D. C.: American Physiological Society, 1962, pp. 51-62.
43. Skelton, H. The storage of water by various tissues of the body. *Arch. Int. Med.*, 40: 140-152, 1927.
44. Stainsby, W. N., and E. M. Renkin. Autoregulation of blood flow in resting skeletal muscle. *Am. J. Physiol.*, 201: 117-122, 1961.
45. Stainsby, W. N. Autoregulation of blood flow in skeletal muscle during increased metabolic activity. *Am. J. Physiol.*, 202: 273-276, 1962.
46. Stewart, G. N. The output of the heart in dogs. *Am. J. Physiol.*, 57: 17-50, 1921.
47. Wiedeman, M. P. Patterns of arteriovenous pathways. In Handbook of Physiology. Section 2, Circulation, vol. II. W. F. Hamilton and P. Dow (eds.). Washington, D.C.: American Physiological Society, 1962, pp. 891-933.
48. Wiedeman, M. P. Dimensions of blood vessels from distributing artery to collecting vein. *Circ. Res.*, 12: 375-378, 1963.
49. Wise, C. S. Fluid Displacement and Pressure Plethysmography. In Methods in Medical Research. vol. 1. V. R. Potter (ed.). Chicago: The Year Book Publishers, Inc., 1948, pp. 182-191.
50. Zierler, K. L. Circulation times and the theory of indicator-dilution methods for determining blood flow and volume. In Handbook of Physiology. Section 2, Circulation, vol. I. W. F. Hamilton and P. Dow (eds.). Washington, D. C.: American Physiological Society, 1962, pp. 585-615.

51. Zuidema, G. D., N. P. Clark, J. L. Reeves, O. H. Gauer and J. P. Henry. Influence of moderate changes in blood volume on urine flow. Am. J. Physiol., 186: 89-91, 1956.
52. Zweifach, B. W. Vasoactive agents in the microcirculation. Fed. Proc., 27: 1399-1402, 1968.